

KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN
COMMISSION OF THE EUROPEAN COMMUNITIES
COMMISSION DES COMMUNAUTÉS EUROPÉENNES

environment and quality of life

Final reports on research sponsored under the
**First environmental research
programme**
(indirect action)

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Directorate-General 'Research, Science and Education'
Environment and Raw Materials Research Programmes

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FOREWORD

The indirect action of the First Environmental Research Programme was approved by the Council on 18th June 1973* for the period 1973-1975 with a total allocation of 6.3 million units of account. It was aimed, together with the direct action carried out in the Joint Research Center, at providing scientific and technical support to the European Community policy on the Environment. The one hundred and twenty seven contracts concluded with institutes and laboratories in the Member States dealt with the following topics :

1. Epidemiological surveys on the effects of air pollution
2. Harmful effects of lead pollution
3. Health effects of micropollutants
4. Ecological effects of water pollutants
5. Remote sensing of air pollution
6. Establishment of a data bank on environmental chemicals

Most contracts ended on 31st December 1975, some were extended beyond that date.

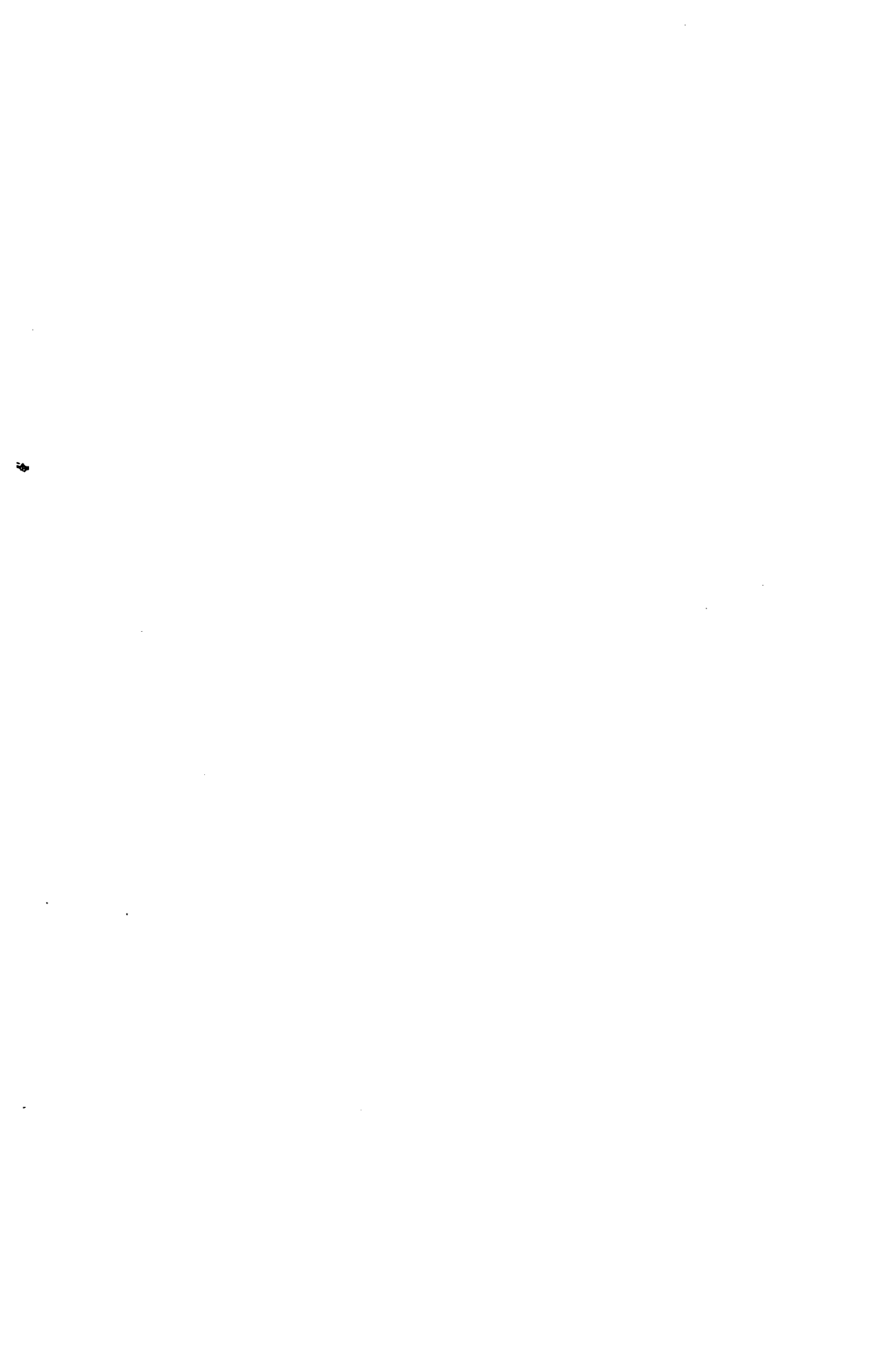
This volume contains the final summary reports on research carried out by each contractor in the form they were submitted. The results of that research are exploited in the implementation of the Community environmental policy especially with regard to

- the objective evaluation of the risks to health and the environment from pollution
- the improvement of pollutant measurements
- the management of environmental information.

The professional staff assigned to the management of the First Environmental Research Programme of the E.C. during 1973 to 1975 were :

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H. Ott)	

* Official Journal of the E.C. n° C 61/1, 28 July 1973.



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COMMISSION OF THE EUROPEAN
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DG XII - Research, Science and
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Joint Research Center
Ispra (Varese)



TOPIC 1 : EPIDEMIOLOGICAL SURVEYS ON THE EFFECTS OF AIR POLLUTION

Contractors : see text

Contracts n° see text

Project leaders : see text

Title of project : Epidemiological survey between air pollution
and respiratory symptoms and disease in children

The survey on health effects of air pollution has been the first example of a coordinated research project carried out jointly in 6 Member States. The institutions, which have participated in the survey, performed the investigations under contract with the C.E.C. on the basis of a common protocol previously prepared by a Group of experts*. These institutions were :

- Inst. Lufthygiene u. Silikoseforschung,
Düsseldorf (D) - contract n° 005-74-1 ENV D
Project leader : Dr. R. Dolgner
- INSERM, Lyon, Paris, Toulouse (F) - contracts n° 010/07/09-74-1 ENV F
Project leaders : Dr. S. Perdrizet,
Dr. C. Rumeau-Rouquette
Prof. P. Bourbon
Dr. J. Bourdeix
- SEMSE, Laboratoire d'Hygiène,
Bordeaux (F) - contract n° 008-74-1 ENV F
Project leaders : Prof. P. Freour,
Dr. J.F. Tessier
- University of Padova (I) - contract n° 011-74-10 ENV I
Project leader : Prof. B. Paccagnella
- UCL - Med. Inst. St. Barbara, Lanaken (B) - contract n° 001-74-1 ENV B
Project leader : Prof. A. Minette
- University of Gent (B) - contract n° 004-74-1 ENV B
Project leaders: Prof. K. Vuylsteek
Dr. A.M. Depoorter
- Dept. of Community Medicine -
St. Thomas Hospital, London (UK) - contract n° 003-74-1 ENV UK
Project leaders : Prof. W.W. Holland,
Dr. Ch. du V. Florey
- Medico-Social Research Board, Dublin (IR) - contract n° 002-74-1 ENV EIR
Project Leaders : Dr. G. Dean,
Dr. D. Keating

* Dr. A. Borlée, Prof. F. Lechat (B) ; Dr. R. Dolgner (D) ; Dr. D. Brille (F) ;
Prof. B. Paccagnella (I) ; Dr. G. Dean (IR) ; Dr. R. Van der Lende (NL) ;
Dr. Ch. du V. Florey, Prof. W.W. Holland (UK)

The 19 study areas selected for the survey were :

3 areas in the Federal Republic of German	Düsseldorf Duisburg Rheidt
5 areas in France	Paris Lyon I Lyon II Lacq Bordeaux
3 areas in Italy	Milano Venezia Ferrara
2 areas in Belgium	Gent Ardennes
3 areas in the United Kingdom	Middlesbrough Stockton Hartlepool
3 areas in Ireland	Dublin Galway Cork

The location of the areas is indicated in the attached map.

Over 20.000 6 to 11 year old schoolchildren were the subject of investigations (~1000/area) during the Spring 1975. The main survey was preceded by a pilot study on 100 subjects in each of the 19 areas. Physical measurements (weight, height and ventilatory lung function) were executed at the schools by personnel previously trained in a joint programme. The same fieldworkers interviewed the parents by using a questionnaire on health and socio-economic status of their children.

All the results of physical measurements and the answers to the questionnaire have been stored into a central computer and checked for error. Three of the participating institutions (St. Thomas, INSERM and Univ. Padova)* are performing the statistical analysis of the data in order to establish a series of possible correlations (e.g. ventilatory function vs. age-sex-area ; symptoms/disease vs. area).

With regard to the atmospheric pollution in the study areas, sulphur dioxide (SO₂) and suspended particulate matter (SPM) were used as indicators and they were measured in the framework of a harmonised programme**. The criteria retained for this programme have

* under the responsibility of Dr. Florey and Messrs Swan, Lellouch, Pesarin.

** Collaboration DG XII and DG V (Messrs Berlin, Price)

led to the installation of additional sampling stations in some areas and of reference stations in each of the 19 areas. The installation of the latter was necessary since SO₂ and SPM were measured by using different analytical methods. The analyses carried out by a central laboratory (Inst. Hygiène et Epidémiologie, Brussels) of strong acidity and black smoke on the samples collected by means of the reference stations provided comparable data on air pollution. The results of these analyses should also permit to establish correlations between analytical methods. The following institutions had the responsibility of the air pollution measurements in the respective area :

- Inst. Lufthygiene u. Silikoseforschung,
Düsseldorf (D) - contract n° 113-75-10 ENV D
Project leader : Dr. A. Brockhaus
- SEMSE, Laboratoire d'Hygiène,
Bordeaux (F) - contract n° 120-75-10 ENV F
Project leader : Mr. J.G. Faugère
- I.R.C.H.A., Paris (F) - contract n° 121-75-10 ENV F
Project leaders : Prof. B. Festy
Melle F. Coviaux
- Comité Départemental du Rhône, Lyon (F) - contract n° 119-75-10 ENV F
Project leader : Mme N. Gally
- INSERM, Toulouse (F) - no contract
Project leader : Prof. P. Bourbon
- Comune di Ferrara (I) - contract n° 123-75-10 ENV I
Project leader : Prof. G.C. Rio
- Lab. Prov. Igiene e Profilassi,
Milano (I) - no contract
Project leader : Dr. G. Rebuzzini
- Ist. Super. Sanità, Roma (I) - contract n° 125-75-10 ENV I
Project leader : Prof.ssa S. Cerquiglini
- Inst. Hygiène et Epidémiologie (B) - contract n° 122-75-11 ENV B
Project leader : Mr. G. Verduyn
- University of Gent (B) - contract n° 130-75-10 ENV B
Project leader : Prof. F.M. Bosch
- Middlesbrough Borough Council (UK) - contract n° 128-75-10 ENV UK
Project leader : Mr. D. Clark
- Medico-Social Research Board, Dublin (IR) - contract n° 129-75-10 ENV EIR
Project leaders : Mr. G.A. Lawlor
Mr. J.F. Nolan
Mr. K. O'Brien
Mr. O'Flaherty

Information on meteorological parameters was also collected in each study area.

The data on SO₂ and SPM concentrations obtained by means of the already existing stations for the years 1973 to 1977 and of the reference stations, installed by the Commission, for the years 1976 and 1977 stored into a central computer are elaborated in order to calculate the exposure levels in the study areas.

A combined analysis of the data on children, air pollution levels and meteorological conditions is at present in progress. The results of such analysis and the conclusions of the survey will be published later.

Publications and oral communications concerning
the European survey :

- | | |
|--|---|
| Ph. BOURDEAU
(on behalf of
the experts
who prepared
the protocol
and of C.E.C.) | Method for a European study on possible effects of air pollution in children. Proceedings Intern. Symposium "Recent advances in the assessment of the health effects of environmental pollution" Paris, June 1974. Published by CEC EUR 5360 pages 263-267, 1975. |
| Ch. du V. FLOREY,
S. PERDRIZET | The European air pollution study, presented at the Joint scientific meeting of Medicine and Social Medicine. Rennes, France, July 1975 |
| J. BOURDEIX,
S. PERDRIZET | Etude européenne de l'influence de la pollution atmosphérique sur les maladies respiratoires de l'enfant. As above. |
| A. BERLIN,
E. DI FERRANTE,
Ph. BOURDEAU,
J. SMEETS | Air pollution measurements in a European epidemiological survey of respiratory ailments in children, presented at the Intern. Conference on "Environmental sensing and assessment", Las Vegas, September 1975 |
| Ph. BOURDEAU et al. | La pollution atmosphérique : objet d'actions communautaires presented at the Symposium of "La recherche en matière de pollution atmosphérique", Les Arcs, France, November 1976 |
| J. COOREMAN | Enquête européenne en milieu scolaire, presented at the INSERM Colloquium "Santé et Environnement" December 1976 |

- L.F. GEPTS,
A. MINETTE
Invloed van ouderlijke rookgewoonten op CARA
symptomen bij kinderen.
Tijdschr. voor Geneeskunde, 32, 1247-1250 (1976)
- L.F. GEPTS,
A. MINETTE
The relationship between host factors of allergic
nature and respiratory symptoms.
Rev. Inst. Hyg. Mines, 32, 28-35 (1977)
- L.F. GEPTS,
A. MINETTE,
I. BORLEE,
A. BOUCKAERT,
B. SPAAS,
M. LECHAT
Prévalence des symptômes respiratoires chez 1659
écoliers vivant dans une zone peu polluée des
Ardennes belges. Acta tuberc. pneumol. belg. (in press)
- W.W. HOLLAND
(on behalf of
project leaders
and C.E.C.)
Air pollution and the health of schoolchildren
in the European Community presented at the Intern.
scientific meeting of the Intern. Epidemiological
Association, San Juan, Puerto Rico. September 1977
- E. DI FERRANTE,
A. BERLIN,
Ph. BOURDEAU,
J. SMEETS
Environmental epidemiology : The European Community
approach as above
-



Epidemiological survey on the relationship between
air pollution and respiratory disease in children

Location of study areas

Contractor : Università di Pisa
Facoltà di Scienze Matematiche,
Fisiche e Naturali
Cattedra di Statistica

Contract n° : 106-75-1 ENV I

Project leader : Prof. R. Saracci

Title of project : Epidemiological investigation of a population
living in the neighbourhood of a vinyl chloride
monomer producing factory

The final report is not yet available, being the statistical
evaluation of results still in progress.

TOPIC 2 : HARMFUL EFFECTS OF LEAD POLLUTION

Contractor : Bundesgesundheitsamt, Inst. für Wasser-,
Boden- und Luft-Hygiene, D - 1000 BERLIN 33

Contract n° : 006-74-6 ENV D

Project leader : Prof. Dr. med. W. SINN

Title of project : Kinetics and metabolism of ingested and
inhaled lead from car exhausts

The final report is not yet available, being the statistical
evaluation of results still in progress.

Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie,
D - 5948 Schmalleberg-Grafschaft

Contract n^o 016-74-1 ENV.D

Project Leader: G. Oberdörster

Title of project: Physiological and physiological behavior
investigations in rats after chronic inhalation
of lead and cadmium aerosols alone and in com-
bination with carbonmonoxide, with special
consideration of the effects on embryos

Rats were continuously exposed to an aerosol containing 1,3 and 10 mg lead/m³, to a combination of 500 ppm carbon monoxide (CO) and 3 mg Pb/m³, and to 0.2 mg cadmium/m³. Rats exposed to uncontaminated air served as controls. The aerosol was generated by an ultrasonic nebulizer and after dilution and mixing with clean air was carried into inhalation chambers, with the dimensions 50 cm x 50 cm x 90 cm. The volumetric flow through the chambers was 80 l/min. The concentration of the lead aerosol was determined gravimetrically, the cadmium aerosol was assayed complexometrically and by atomic absorption spectrometry. The median aerodynamic diameters of the lead and cadmium aerosols were in the order of 1 µm and 0.3 µm, respectively, σ_g being 1.7. Carbon monoxide was obtained from gas cylinders. Its flow into the inhalation chambers was controlled by capillaries. The CO concentration in air was determined with an infrared absorption spectrometer (URAS).

Pregnant and nonpregnant rats were exposed continuously for 21 days to the lead aerosols and to the combination of lead and CO. The effects were measured in adults and fetuses. At the two lower aerosol concentrations (1 and 3 mg Pb/m³) the fetal blood lead values exceeded those of the mothers. Active transport mechanisms of the placenta were considered to be responsible for these differences. A decrease of the fetal blood lead level below the maternal level in the high exposure group was explained by an

increasing storage capacity of the fetal livers with increasing lead doses. At the low aerosol concentration (1 mg Pb/m^3) the ratio of lead concentrations of maternal livers to fetal livers was 2.19 : 1 on a ng per gram wet weight basis. At an aerosol concentration of 3 mg Pb/m^3 , the ratio decreased to 1.81, and at the high concentration of 10 mg Pb/m^3 , the ratio was only 1.36.

Lead liver values of the pregnant animals exceeded those of the nonpregnant animals, irrespective of the aerosol concentration. These higher lead liver values in pregnant animals were accompanied by elevated blood lead values at the high aerosol concentration only. As the low aerosol concentration resulted even in higher blood lead levels of the nonpregnant animals, probably an altered pharmacokinetic behavior of lead during pregnancy as well as an increased ventilation rate must be responsible for the increased lead liver concentrations in pregnancy. Additional inhalation of 500 ppm CO to 3 mg Pb/m^3 lowered the liver storage capacity of the adult animals and elevated their blood lead levels. In the fetus, additional CO inhalation raised the liver lead values.

The activity of the fetal δ -aminolevulinic acid dehydratase (ALA-D) was less inhibited by lead than the maternal activity. Furthermore, the degree of inhibition was highly reduced in the fetuses by additional CO-inhalation, whereas in adult animals inhalation in accordance with epidemiological data. Therefore, it is concluded, that the mode of plumbic inhibition of the ALA-D activity differs in fetuses from that in adults. Furthermore, the adaptation to the inhibition of the ALA-D by de novo synthesis of this enzyme was less pronounced in fetuses than in adult rats. The high lead aerosol concentration reduced hematocrit and body weight of the fetuses, but it did not influence these parameters in adult rats, thus pointing to a higher lead sensitivity of the fetal than the adult organism. A stronger inhibition of the ALA-D activity in dams than in nonpregnant animals possibly indicates a higher susceptibility to lead in pregnancy.

Furthermore male rats were exposed for 66 days to an aerosol containing 0.2 mg Cd/m^3 . In these animals no decrease of hemoglobin and hematocrit was observed, while male rats showed lowered hemoglobin and hematocrit levels and comparable cadmium concentrations in the organs after 52 days of dietary cadmium of 25, 50 and 100 ppm in drinking water. From these results the conclusion can be drawn that anemia is a sensitive indicator only after oral administration of cadmium but not after inhalation uptake.

The chronic effects of lead aerosols alone and in combination with CO on learning capability and memory and normal behavior in rats was investigated. The concentrations were 10 mg Pb/m^3 and $3 \text{ mg Pb/m}^3 + 500 \text{ ppm CO}$, respectively, an additional group was exposed to 500 ppm alone. In an elevated maze both time and errors were measured when the rats tried to find their way through the maze. The open field test was used to measure spontaneous activity and exploration behavior.

An impaired memory function was only observed in rats which were exposed to Pb immediately after delivery. Exposure to Pb beginning at the age of 6 weeks resulted in a quite normal behavior in the maze test. Exposure to CO decreased the motility of the rats, they exhibited longer runs in the maze irrespective of age. Additional Pb exposure enhanced this effect. In the open field test juvenile rats showed an increased environmental exploration behavior after lead exposure, similar to hyperactivity. CO alone decreased the motility in this test, and this effect was increased by additional lead exposure, quite in contrast to lead alone. Obviously, inhalation exposure to lead leads to altered CNS-function only when the exposure started shortly after birth.

List of publications

- E. Prigge, H.P. Baumert and H. Muhle
Effects of Dietary and Inhalative Cadmium on Hemoglobin and Hematocrit in Rats.
Bull. Environ. Cont. Tox. 17, 585-590, 1977
- E. Prigge, H.P. Baumert, D. Hochrainer and G. Oberdörster
Effects of Lead Inhalation Exposures Alone and in Combination with Carbon Monoxide in Nonpregnant and Pregnant Rats and Fetuses. I. Distribution of Lead in Blood and Liver.
Zbl. Bakt. Hyg., I. Abt. Orig. B., in press
- E. Prigge and J. Greve
Effects of Lead Inhalation Exposures Alone and in Combination with Carbon Monoxide in Nonpregnant and Pregnant Rats and Fetuses. II. Effects on δ -Aminolevulinic Acid Dehydratase Activity, Hematocrit and Body Weight.
Zbl. Bakt. Hyg., I. Abt. Orig. B., in press

Contractor : Universität des Saarlandes

Contract n^o 018 - 74 - 1ENVD

Project Leader : Professor Dr. Hermann Josef Haas

Title of project : Lead(II)-ions And Their Effect on The
 Metabolism of Halogens

Our studies were based on the observation that in vicia faba, lead(II)-bromide (developed from lead-tetraalkyls and alkyl-bromides) from motor vehicle exhaust gases caused inhibitions of mitosis, changes in chromosomes, and leaf anomalies (H.J.Haas and W.Paulini, unpublished). Similar changes were noted during the exposure of vicia faba to 5-bromouracil, during the course of which this mutagen was incorporated into DNA instead of thymine (W.F.Haut and J.H. Taylor, 1967).

This led to the establishment of the following hypothesis: The changes generated by lead(II)-bromide in vicia faba are due to uracil reacting with lead(II)-bromide and forming 5-bromouracil. However, it is also possible that the formation of 5-bromouracil-containing DNA is caused by bromination of uracil-containing RNA decomposition products (e.g. uridine or uridine 5'-monophosphoric acid) or by incorporation of bromine into RNA pre-stages (e.g. orotic acid).

Should one or more of these hypotheses prove to be right, the changes noted could be attributed to bromide ions. In favour of this hypothesis is the fact that lead(II)-ions do not affect vicia faba in the absence of bromide ions. However, changes in vicia faba call for the presence of lead(II)-ions since potassium bromide does not cause any mutations in the absence of lead(II)-ions (H.J.Haas and W.Paulini, unpublished). Thus, the changes noted might be explained by a synergetic reaction of bromide and lead(II)-ions.

Our studies were conducted in an attempt to establish the

following in vitro: Is the formation of 5-bromo derivatives by uracil and/or its derivatives caused by bromide ions? If so, what is this process conditioned on, and does it require the presence of lead(II)-ions?

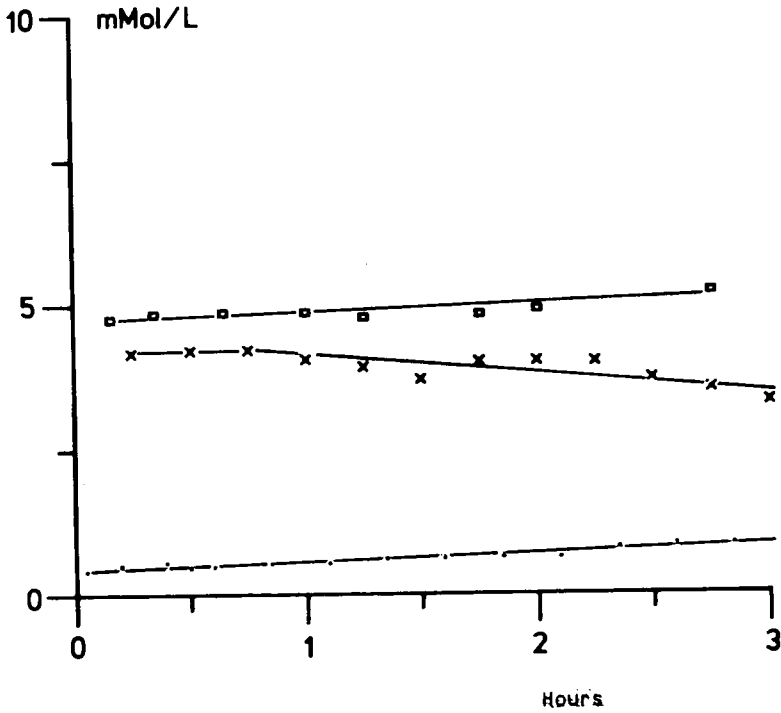
Our studies revealed that in diluted solutions treated at 37°C with bromide and lead(II)-ions, uracil and orotic acid were actually converted into their respective 5-bromo derivatives, that uridine released only a small amount of its 5-bromo derivative, and that UMP did not brominate at all (or revealed traces of bromination at the most). The incorporation of bromine was found to call for an oxidizing agent, such as oxygen or hydrogen peroxide. Since these agents are also present in cells, they might take an active part in the formation of 5-bromo derivatives in vivo. The finding that lead dioxide can take the place of hydrogen peroxide or oxygen might be interpreted by the effect of lead ions being connected with a change of its valence. In 0,01M solutions at pH 3, 7 and 11, orotic acid was converted into 5-bromorotic acid by equimolar amounts of lead(II)-bromide and lead dioxide. Figure 1 shows the period of time required for the reaction at pH 3. So far, the highest bromination rate noted for uracil has been approximately 50%. This rate was found in 0,01M solutions treated at pH 3 with equimolar amounts of lead(II)-bromide and lead dioxide. (For period of time elapsed see figure 1). Under the same conditions, the amount of 5-bromouridine was only 8% due to the fact that 65% of the uridine employed underwent oxidative changes caused by lead dioxide. Uracil was also brominated with equimolar amounts of potassium bromide/hydrogen peroxide/lead(II)-acetate; amount of 5-bromouracil gained: 25% after 3 hours of treatment in 0,01M solutions at pH 2,4. Since uracil was also found to brominate with equimolar amounts of potassium bromide/hydrogen peroxide in the presence of an amount of lead(II)-acetate which was 20 times smaller than that of potassium bromide/hydrogen peroxide, it is assumed that lead(II)-ions have a catalytic effect.

In favour of this assumption is the fact that equimolar amounts of potassium bromide/hydrogen peroxide do not affect uracil in the absence of lead(II)-ions. (However, an excess amount of hydrogen peroxide resulted in uracil revealing traces of bromination even in the absence of lead(II)-ions).

In order to check all test series (with a total of 50-100ml) for possible bromination, there were equal portions (consisting of 5 ul, as a rule) taken and transmitted on cellulose plates by means of thin-layer chromatography. After the development of chromatograms, the spots pertaining to crotonic acid, uracil, uridine, UMP, and their 5-bromo derivatives, respectively, were directly evaluated (with the VIS-UV-2 chromatogram analyzer, Fa. Farrand-Kontron) by means of adequate calibration curves. During this process, there were defined amounts of the substances to be determined carried on each plate. Furthermore, in the case of a preparative experiment with uracil bromination with lead(II)-bromide/lead dioxide, the 5-bromouracil developed was separated from cellulose by means of column chromatography and, by mass- and IR-spectrometry, melting point, and mixed melting point, identified with an authentic sample (Fa. Fluka). Our findings suggest that the changes in *vicia faba* caused by lead(II)-bromide did, in fact, result from a synergetic reaction of lead- and bromide ions during the course of which bromide ion in the form of 5-bromouracil was incorporated into genome.

Figure 1: Formation of 5-bromo derivatives of orotic acid, uracil, and uridine during treatment with equimolar amounts (0,01 Mol/L each) of lead(II)-bromide and lead dioxide at pH 3 and 37°C.

x—x 5-bromoorotic acid
 □—□ 5-bromouracil
 .— . 5-bromouridine



Contractor: Prof. Dr. E. Schnepf - University of Heidelberg
Contract n° 019-74-1ENV-D

Project Leader: Prof. Dr. E. Schnepf

Title of project: Wirkung von Blei auf die Feinstruktur
pflanzlicher Zellen am Beispiel des Phyto-
flagellaten *Porterioochromonas*

We studied the influence of Pb^{++} , and of the antiknock compounds tetraethyl lead (TEL) and triethyl lead TriEL on growth, morphogenesis, division and ultrastructure of the chrysophycean flagellate *Poterioochromonas malhamensis*. The accumulation of lead by the alga depends on cell liter, concentration of lead and incubation time. Growth is stopped by 1m M Pb^{++} , 0.5 m M TEL and 0.01 m M TriEL. The algae react after treatment with each of the three substances by the formation of loricae with shorter stalks; TEL and TriEL often induce stalkless loricae. After longer incubation with TEL and TriEL giant cells arise. They either contain many nuclei or giant, obviously polyploid nuclei. Studies with many microtubule-affecting agents suggest that the organic lead compounds also interfere with microtubules. Aeration and light enhance the effects of TEL. Presumably, TEL is decomposed photolytically and TriEL is the toxic agent proper. It was not possible to decrease the toxicity of the organic lead compounds by several substances. The effects of the lead compounds on the ultrastructure of the *Poterioochromonas* cells are studied in detail. Cortical microtubules are present when other cell organelles, especially mitochondria, nuclei and dictyosomes show severe pathological alterations. In so far TriEL cannot be a very specific microtubule agent though its toxicity presumably is mainly caused by effects on microtubules. It is an open question whether this is also true for the toxic effects of the organic lead compounds on man.

List of publication, prepared within the contract:

1. G. Röderer: Die Wirkung von Blei auf Wachstum, Hüllenmorphogenese und Ultrastruktur von *Poterioochromonas stipitata*. Diplom-Arbeit, Fak. Biologie Univ. Heidelberg 1975
 2. G. Röderer: Induction of giant, multinucleate cells with tetraethyl lead. *Naturwiss.* 63, 248-1976
 3. E. Schnepf, G. Deichgräber, G. Röderer and W. Herth: The flagellar root apparatus, the microtubular system and associated organelles in the chrysophycean flagellate, *Poterioochromonas malhamensis* Peterfi (syn. *Poterioochromonas stipitata* Scherffel and *Ochromonas malhamensis* Pringsheim) *Protoplasma* 92, 87-107 (1977)
 4. Röderer, G.: Cytologische Untersuchungen zur Wirkung von anorganischen und organischen Bleiverbindungen auf *Poterioochromonas malhamensis*. Dissertation Fak. Biologie Univ. Heidelberg 1977
 5. G. Röderer and E. Schnepf: Tetraethyl lead and triethyl lead inhibit cytokinesis of the chrysophycean flagellate *Poterioochromonas*. *Naturwiss* (in press)
- Other publications are in preparation.

Contractor: Medizinisches Institut für Lufthygiene und
Silikoseforschung an der Universität Düsseldorf

Contract n^o 062 - 74 - 1 ENVD, Project 1

Project leader: Prof. Dr. med. H.-W. Schlipkötter

Title of project: Animal experiments on the effect of inhaled
lead compounds on the lung

Inhalation of lead oxide in concentration of 70 - 150 $\mu\text{g}/\text{m}^3$ impairs the structure and the function of the lung in several investigated parameters.

The ultrastructure of the alveolar macrophages and the pneumocyte type I indicate injurious effects on the lung parenchyma. The pneumocyte type I exhibits also an increased ^3H -thymidine labelling index. This result can be explained by elevated turnover of cells at the alveolar wall (table 1).

In two chronic 15 weeks-experiments with alternated inhalation of lead oxide (82 and 168 $\mu\text{g}/\text{m}^3$; 5 h/day) and titan dioxide aerosols (19 and 28 mg/m^3 ; 3 h/day) lung clearance was impaired by 33 % to 39 % (table 2) (Bruch et al. 1975).

In a second set of experiments the effects of lead chloride were tested with a concentration of 100 and 20 $\mu\text{g}/\text{m}^3$. No significant differences between PbCl and control could be shown in respect to lung retention of TiO_2 (table 3). In general PbCl induces far less damage to the lung parenchyma than PbO . Probably the phenomenon is due to the different solubility of the compounds: PbCl is more soluble, so that the lead-concentration may drop rapidly in the deep lung parenchyma. On the other hand, PbO remains comparatively longer in the lung tissues.

In addition the influence of lead aerosols on viral infection as well as on bacterial clearance of the pulmonary system of mice has been tested.

Pb ($150 \mu\text{g}/\text{m}^3$) does not alter the mortality rate of mice, when intranasally inoculated with parainfluenza virus. The DL_{50} after 7 days inhalation of PbO and a standardized virus infection happened at day 3-4, corresponding to the animals of the control group.

On the other hand animals inhaling an aerosol of *Serratia marcescens* after exposure to PbCl ($18 \mu\text{g}/\text{m}^3$) retain at the end of 9 weeks treatment significantly more bacteria than control animals. The results show that the model used, i.e. bacterial lung clearance, is a sufficient sensitive method to detect early pulmonary impairment due to low PbCl-concentration (Schlipkötter et al. 1977).

Conclusions in regard to the possible effects of actual air-lead-concentrations should be drawn very carefully. Apparently the watersolubility of the compounds and the cumulative character of the lesions seem to be important factors for the hygienic qualification of damaging effects on the lungs due to lead compounds.

Exp. group	No. labelled type I-cells/ 1000 cells	p	No labelled type I-cells/ cm^2 inner lung surface	p
Control	3.2		$1.5 \cdot 10^5$	
PbO (14 days, $150 \mu\text{g}$)	15.5	< 0.001	$9.1 \cdot 10^5$	< 0.001

table 1

	Inhalation time weeks	Treatment		Retention		
		TiO ₂ -conc. mg/m ³	Pb-conc. /ug/m ³	TiO ₂ mg/lung		Increase of retention: percent.of control
				\bar{x}	s	
I c	15	18,8	0	0.40	0.1	
I PbO	15	18.8	82	0.53	0.1	33 %
II c	15	27.7	0	0.69	0.18	
II PbO	15	27.7	168	0.96	0.29	39 %

table 2

Treatment		Retention	
		TiO ₂ \bar{x}	mg/lung s
Control		0.47	0.13
PbCl	20 /ug/m ³	0.48	0.17
PbCl	100 /ug/m ³	0.52	0.11

table 3

Publications

Bruch, J., A. Brockhaus et W. Dehnen:
 Action du plomb sur les macrophages alvéolaires in vivo.
 Rev. franc.Malad.Respir., 2, Suppl. 1, 139 - 142 (1974)

Bruch, J., A. Brockhaus and W. Dehnen:

Local effects of inhaled lead compounds on the lung.

In: Tagungsberichte Internationales Symposium "Neueste Erkenntnisse in der Beurteilung der gesundheitlichen Folgen der Umweltverschmutzung", Paris, June 24 - 28, 1974. -

Luxembourg: Commission of the European Communities, Directorate General Scientific and Technical Information and Information Management, 781 - 791 (1975)

Schlipkötter, H.-W., G.H. Krause, R. Stiller-Winkler und A. Brockhaus:

Die Beeinflussung der Infektionsresistenz durch staub- und gasförmige Luftverunreinigungen. In press.

Contractor: Medizinisches Institut für Lufthygiene und
Silikoseforschung an der Universität Düsseldorf

Contract n^o 062 - 74 - 1 ENVD, Project 2

Project leader: Prof. Dr. med. H.-W. Schlipkötter

Title of project: Behavioral toxicity of lead in rats after
pre- and postnatal exposure

In order to assess neurobehavioral deficit after longterm treatment with inorganic lead, Wistar-rats were exposed to lead through lead-acetate in the following developmental sequence: Prenatally via their lead-fed dams, neonatally through their lead-treated dams' milk, and later on via their own diet containing 1.38 mg lead-acetate/kg diet. Preliminary feeding studies had shown this concentration to produce the desired equilibrium blood-lead level (PbB) of about 30 $\mu\text{g}/100\text{ ml}$ in rats. 20 lead-treated animals were compared with 20 age-matched controls.

Behavioral testing

Male offsprings only were subjected to behavioral testing when being between about 100 and 200 days old. An open field-test was used for the assessment of complex exploratory activity. On five consecutive days the animals' behavior in the circular open field was videotaped for two minutes, and scored later on in a blind fashion for "ambulation" (locomotor activity), "rearing" (vertical activity), "grooming" (cleaning activity), and "emotionality" (deposition of fecal boluses). Accuracy of scoring was determined by measuring inter-rater-agreement, which exceeded 0.90 (coefficient of correlation) for these behavioral categories.

A LASHLEY-type visual discrimination learning task was used for the assessment of learning performance. Two visual patterns were selected from CARSON et al. (1974) to represent

extremes on a difficulty dimension. The "easy" task consisted of discriminating between vertical and horizontal stripes, the "difficult" one of discriminating between large and small circles. Animals were kept on 80 % of their normal amount of food intake during the learning part of the experiment, and were reinforced by food when choosing the correct pattern. Details of the procedure have been described elsewhere (WINNEKE et al. 1976 a, b, 1977). Duration of learning was restricted to 12 days for the easy pattern, and to 27 days for the difficult one.

Results

(1) Blood-lead concentrations (PbB)

Blood samples were taken retroorbitally from female rats 10 days before mating and 10 days after weaning, and from their offspring at the age of about 16 and 190 days. The samples were pretreated with strong oxidizing acids, the lead extracted into organic solution by chelating agents, and determined by flameless atomic absorption photometry. PbBs of lead-treated dams increased from about 24 to 31 $\mu\text{g}/100\text{ ml}$ during pregnancy and suckling periods, those of lead-fed offspring did not exceed 30 $\mu\text{g}/100\text{ ml}$ on the average; PbBs of control-animals remained below 6 $\mu\text{g}/100\text{ ml}$ throughout the experiment.

(2) Open field-activity

Lead-treated animals as compared to their age-matched controls appeared overactive. This was true for the ambulation-score, describing locomotor activity ($p < 0.01$), for the rearing-score describing vertical activity of the animals ($p < 0.01$), as well as for the grooming-score describing cleaning activity ($p < 0.05$). No significant difference due to lead-treatment could be detected for the emotionality-score, as characterized by the number of fecal boluses.

(3) Learning performance

The influence of lead-treatment on learning was clearly different for the two patterns used. For the easy pattern, vertical vs. horizontal stripes, no significant difference between treatment-groups was found. Lead-treated animals did learn the easy discrimination-problem at about the same rate as did their age-matched controls. For the difficult pattern, large vs. small circles, however, a clearcut lead-influence did occur: 8 out of 10 control-animals did learn the difficult discrimination task within the 27 days learning period, whereas only one lead-treated animals did. This difference was statistically significant ($p < 0.01$).

(4) Body-weight

The body-weight of the lead-treated animals, taken as a group, was 295.1 g (± 46.9 g), that of control-animals only 270.5 g (± 53.6 g). This slight superiority of lead-treated animals was significant ($p < 0.01$), if the additional variability due to the different age-groups was taken into account.

Conclusions

Although lead-treated animals, having been exposed to elevated PbB-levels between about 24 and 31 $\mu\text{g}/100$ ml throughout their lifetime, did not exhibit overt symptoms of lead-poisoning, as e.g. tremor, seizures or loss of appetite, they did, nevertheless, demonstrate covert signs of neuropsychological dysfunction, namely hyper- or overactivity and impaired learning performance. One or the other of these symptoms have been described by others, too, but both these symptoms together have not been observed in the same animals before. CARSON et al. (1974) described impairment of learning performance in

sheep after prenatal exposure to maternal PbB of 34 $\mu\text{g}/100\text{ ml}$. The body burden of the animals of our study was even lower.

Although it is clearly impossible to determine from the data presented here, whether lead-induced neuropsychological dysfunction represents a long-lasting or even a lifelong deficit, and whether the critical periods of lead-influence are limited to prenatal and/or neonatal developmental stages, it must be emphasized that slightly elevated blood-lead levels have again been shown to be causative of marked CNS-dysfunction in rats, and that PbBs around or even below 30 $\mu\text{g}/100\text{ ml}$ are sufficient for this damage to occur.

Publications

Carson, T.L., G.A. van Gelder, G.L. Karas, and W.B. Buck:
Slowed Learning in Lambs Prenatally Exposed to Lead. Arch.
Environ.Health 29, 154 - 156 (1974)

Winneke, G., A. Brockhaus und R. Baltissen:
Diskriminationslernen und Open Field-Verhalten prä- und post-
natal bleibelasteter Ratten. 18. Tagung experimentell arbei-
tender Psychologen. Bochum 12. bis 14.4.1976. Vortrag

Winneke, G., A. Brockhaus, R. Baltissen und N. Grieser:
Neuropsychologische Untersuchungen bei Ratten nach prä- und
postnataler Bleiexposition. 6. Arbeitstagung der Deutschen
Gesellschaft für Hygiene und Mikrobiologie e.V., Mainz,
27. bis 29.9.1976. Vortrag

Winneke, G., A. Brockhaus und R. Baltissen:
Neurobehavioral and Systemic Effects of Longterm Blood Lead-
Elevation in Rats. I. Discrimination Learning and Open Field-
Behavior. Arch. Toxicol. 37, 247 - 263 (1977)

Contractor: Battelle-Institut e.V., Frankfurt am Main

Contract No.: 063-74-1 ENV-D

Project Leader: Dr. H. J. Kinkel

Title of Project: Experimental Investigations into the
Effect of Lead in the Atmosphere on
Infections of the Respiratory Organs,
Exemplified by the Influenza Infection
in Mice

Problem and Objective

Very little is known about the influence of environmental toxicants on the susceptibility to infections produced by viruses or bacteria. There are reasons to assume, however, that physiological effects of environmental pollution results in a reduced resistance to infective agents. One of the main diseases of the respiratory system is influenza, which afflicts in particular densely populated areas, where there is a high degree of air pollution particularly with lead. Battelle-Frankfurt was therefore commissioned to investigate the influence of lead acetate inhalation on the course of a virus infection of the respiratory organs of mice.

The influenza virus reproduces in the ciliated epithelium cells of the human respiratory system and, in the case of experimental infection, in the same cells of the mouse. After adaption it shows there the same bronchopneumotropism as in humans; hence this virus-mouse model is accepted as a representative model and thus provides a very sound basis for investigating the present problem.

Material and Method

Experimental animals: male SPF mice (NMRI). Influenza virus: Strain A-Asia supplied by Behringwerke, Marburg. Lead: PB acetate (Merck) - specific lead effect. Inhalation chambers: Niessen^{*)} modified chambers. Particle counter: cascade impactor (Kontron, Munich). Determination of the blood lead levels: by atomic spectrum photometry. Assessment criteria for the effect of lead: delta-amino-levulinic acid dehydratase activity in the blood (ALA-D), hemoglobin (Hb), hematocrit (HK), delta-amino-levulinic acid in the urine (ALA-U), coproporphyrin in the urine (CPU).

The animals (nine per group) were exposed to a relatively large dose of lead for a short time (60 mg/m^3 for four hours) or to a small dose for 28 days (1.5 mg/m^3 or 5 mg/m^3 for four hours daily) and then infected with the influenza virus in five different concentrations. The mean lethal dose (LD_{50}) was determined according to Spearman-Kärber, 14 days after infection.

Results and Discussion

The exposure to lead was clearly reflected in the correspondingly higher blood lead level, the reduction in δ -ALA-D and the increase in ALA-U.

^{*)} Niessen et al.: Arch. f. Toxikol. 20 (1963) 44

The virus infection changed the white blood count. Three to eight days after infection there was a statistically significant dose-dependent increase in polymorphonuclear neutrophils, while the proportion of lymphocytes had decreased accordingly. Two weeks after infection these values returned again to the original values. The percentages of all the other cell types remained constant.

In both types of experiments, those with short-time lead inhalation and those with lead inhalation over 28 days, the mice that were exposed to lead died earlier than the controls.

The statistically insignificant increase in the mortality rate was particularly pronounced in the 28 day experiment and suggests that the virus infection manifests itself more quickly after lead inhalation. However, there was no corresponding tendency in the LD₅₀ values or the white blood count, although individual values of corresponding dose groups obtained through statistical analysis were found to be statistically significant. It should be noted, however, that in this analysis the respective individual values may vary greatly.

The experiments were supplemented by an experiment where the virus infection preceded lead inhalation (25 mg/m³ lead acetate for four hours each on five consecutive days). Again, the animals exposed to lead died earlier. The LD₅₀ value for the collective "virus + Pb" was lower than that for the collective "virus".

Conclusion

The experiments performed so far thus represent a first contribution to a solution of the complex problem under consideration. It cannot be ruled out that lead inhalation has a synergistic effect on infections of the respiratory organs, however, additional experiments are necessary to provide a sound statistical basis for the results relating to the influence of exposure to lead on virus infections. The following should be taken into consideration for further investigations on this problem:

- lead inhalation in different particulate or aerosol forms,
- lead inhalation before and during virus infection,
- extension of the period of exposure with reduction of the lead concentration,
- increasing the number of animals per experimental group.

Contractor: Prof. Dr. med. H. Valentin
Contract No.: 070/74/1 ENVV
Project leader: Prof. Dr. med. H. Valentin

Title of project: The influence of defined stresses
on lead metabolism

Summary and Conclusions

In recent years standards for ecological and occupational lead-exposure were established. On these occasions also various mechanisms of lead mobilization have been discussed. They still represent an important factor of uncertainty. Theoretically a release of lead in particular by altering bond conditions in bone and in soft tissues appears possible. Up to now clinical examinations had contradictory results. As factors of influence that may cause a mobilization of depot-lead are considered: chronic diseases, disturbances of the acid-base balance, feverish illnesses, medications, as well as extreme psychical and physical stresses. In former examinations we could not ascertain any significant variations of lead-metabolism under experimental fever. Patients suffering from malignant tumors, acidoses, skull-brain-traumata, apoplectic strokes, Marcumar[®]-treatment and renal insufficiency were examined primarily on alterations of their blood-lead levels. All these collectives showed no deviations from the

normal. Follow up-investigations, carried out to an extent possible, also displayed no alterations, larger than the analytical error of the blood-lead determination.

The influence of dietetical factors, appearing in acidifying and in alkalizing diets was tested on normals and on extremely lead-exposed workers. Here as well no variations of lead metabolism were visible. Tests on soldiers before and after a long distance march also did not result in significant variations of blood-lead level.

From these results may be concluded that chronic diseases, short and longerlasting disturbances of the acid-base balance, extreme changes of diet, Marcumar[®]-treatment as well as intensive physical stress under conditions as described lead to no or only to irrelevant lead mobilizations in normal adults. At all events the variations range under the analytical tolerance of determination of lead in blood. The imagination of a significant mobilization of lead from depots under extreme conditions appears to be in need of revision, at least as far as adult normals are concerned. The results may not be transferred as a whole in persons occupationally exposed to lead or on children. For this further research is necessary.

Publications prepared with in the contract:

SCHALLER, K.H.:

Zur analytischen Zuverlässigkeit der Blutblei- und der Delta-Aminolaevulinsäuredehydratase-Aktivitätsbestimmung im ökologischen Bereich.

Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene.

G. Fischer Verlag, Stuttgart (in press)

BOST, H.-P., R. LINDNER, K.H. SCHALLER, H. VALENTIN:

Die Beeinflussung des Bleistoffwechsels durch erhöhte Körpertemperaturen.

Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene.

G. Fischer Verlag, Stuttgart (in press)

SCHIELE, R., K. H. SCHALLER:

Untersuchungen zur "Bleispezifität" der Delta-Aminolaevulinsäure-Dehydratase

(ALA-D) der Erythrozyten.

Jahresbericht der Deutschen Gesellschaft für Arbeitsmedizin, 1976 pp. 163

A.W. Gentner Verlag, Stuttgart

SCHIELE, R., H.W. WAGNER, C. KRAUSE:

Die freien Erythrozytenporphyrine (FEP) als Wirkungsparameter und seine Wertigkeit im Vergleich zu anderen Belastungskriterien.

Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene.

G. Fischer Verlag, Stuttgart (in press)

Contractor: Institute of Occupational- and Social-Medicine of
University Erlangen-Nürnberg, FRG

Contract No.: 070/74/1 ENV D

Project leader: Prof. Dr. med. H. Valentin

Title of Project: The influence of defined stresses on lead metabolism
in man

Als besonderes Risiko der Anreicherung von Blei in Knochen und anderen Körpergeweben wird von verschiedenen Arbeitsgruppen noch heute eine potentielle Mobilisierung durch verschiedene exogene und endogene Einflüsse diskutiert. In erster Linie wird dabei an folgende Faktoren gedacht:

1. fieberhafte Erkrankungen,
2. Störungen des Säure-Basen-Haushaltes,
3. außergewöhnliche physische und psychische Belastungen,
4. medikamentöse Einflüsse,
5. verschiedene chronische Erkrankungen.

In der vorliegenden Studie wurde eine Auswahl derartiger Einflüsse in ihrer Wirkung auf den Blutbleispiegel untersucht. Bei der Versuchsdurchführung wurden sowohl experimentelle als auch praktisch-klinische und arbeitsmedizinische Bedingungen berücksichtigt.

UNTERSUCHUNGEN UND ERGEBNISSE

1. Die Beeinflussung des Bleistoffwechsels durch erhöhte Körpertemperaturen
Sechs klinisch gesunde Männer und vier Frauen wurden innerhalb von 8 Tagen dreimal einer experimentellen Fieberbelastung durch einmalige i.v. Injektionen von Pyrifin^R unterworfen. Es setzte 2 bis 4 Stunden nach der Injektion ein und hielt 7 bis 40 Stunden an. Die erreichten maximalen Temperaturen lagen zwischen 38,1 und 40,5°C. Gemessen wurden der Blutbleispiegel (Pb-B), die Erythrozyten-bezogene Blutbleikonzentration (Pb-E), die Delta-Aminolaevulinsäure-Dehydratase-Aktivität (ALA-D) und die Konzentration der freien Erythrozytenporphyrine (FEP). Im Urin wurde die Bleiausscheidung in 24 Stunden (Pb-U) bestimmt. Alle Meßwerte lagen im Normbereich. Varianzanalytisch zeigte keiner der gemessenen Parameter signifikante Veränderungen.

2. Störungen des Säure-Basen-Haushaltes - Diätetische Umstellung bei Normalpersonen und Personen mit beruflicher Bleiexposition

Im Modellversuch wurde das Verhalten des Pb-B und der Pb-U unter säuernder und alkalisierender Nahrung und unter zusätzlicher tgl. Gabe von 15ml 2%iger Phosphorsäure bzw. 10g des Handelspräparates Uralyt-U (Kaliumnatriumcitrat) festgestellt.

2.1.1 Normalpersonen

5 weibliche und 5 männliche gesunde, freiwillige Versuchspersonen nahmen in 2 Wochen für jeweils 4 Tage säuernde und nach einem Wochenende mit Normalkost alkalisierende Diät ein. Unterstützt wurden die Diäten durch die Verabreichung der genannten Chemikalien. Während sich die pH-Werte im Urin und Blut signifikant änderten ergab sich für die tgl. Bleiausscheidung und den Pb-B-Spiegel keine Abhängigkeit von der Kostform. Dies gilt auch für die tgl. gemessene ALA-Ausscheidung im Urin.

2.1.2 Personen mit beruflicher Bleiexposition

14 freiwillige männliche Angehörige einer Bleischmelze wurden wie oben beschrieben einer säuernden Diät ausgesetzt. Die Bleiexposition dieser Personen dokumentierte sich durch Pb-B-Spiegel im Bereich von 50 bis 86 µg/100ml. Auch bei diesem Kollektiv ließen sich keine signifikanten Veränderungen der Pb-B-Spiegel und der tgl. Bleiausscheidung mit dem Urin feststellen.

2.2 Personen mit Niereninsuffizienz

20 Patienten mit chron. Niereninsuffizienz wurden untersucht. Die Ergebnisse der Querschnittsuntersuchung zeigten, daß die Pb-B-Spiegel aller Patienten im Normbereich lagen. Dies gilt auch für die indirekten biochemischen Belastungsparameter, wie ALA-D und die FEP.

2.3 Personen mit akuter Acidose, mit Schädel-Hirntraumata und mit Apoplexien

Die Pb-B-Spiegel von 4 Patienten mit einer schweren Acidose, von 2 Patienten mit akut aufgetretener Hemiplegie bzw. Tetraplegie, von 5 Patienten mit epiduralen bzw. subduralen Hämatomen und von 1 Patienten mit Impressionsfraktur des Schädels wurden über einen längeren Zeitraum atomabsorptionsspektrometrisch gemessen. Die Pb-B-Spiegel lagen durchwegs im Bereich der für Normalpersonen gültigen Werte. Auch während des Untersuchungszeitraums ließen sich keine statistisch gesicherten Veränderungen feststellen.

3. Physische und psychische Belastungen

3.1 Physische Belastung

31 gesunde Versuchspersonen absolvierten einen Leistungsmarsch über 25 km. Vor und nach Beendigung der Belastung wurde der Blutbleispiegel gemessen. Die Meßwerte lagen durchwegs in dem für unsere Methode gültigen Normbereich. Nach Beendigung des Marsches zeigten sich keine signifikanten Veränderungen des Pb-B-Spiegels.

3.2 Psychische Belastung

Untersucht wurden 10 Lokomotivführer die Hochgeschwindigkeitsfahrten bis zu 250 km/h durchführten. Die Pb-B-Spiegel wurden sowohl vor und nach der Fahrt als auch in der Funktion als Fahrer oder Beifahrer gemessen. Die Werte lagen im Normbereich und zeigten keine signifikanten Veränderungen unter der Belastung.

3.3 Untersuchungen bei Schwangeren

Eine Schwangerschaft kann als wohl definierter, zeitlich begrenzter Stress angesehen werden. Ob diese Belastung Anlaß zu Veränderungen des Pb-B-Spiegels ergibt wurde durch einen Vergleich eines Kollektives von schwangeren Frauen am Ende der Schwangerschaft mit einem Kollektiv von nicht schwangeren Frauen (n=31) desselben Gebietes, Alters und Milieus untersucht. Beim statistischen Vergleich ließ sich kein signifikanter Unterschied feststellen.

4. Medikamentöse Einflüsse

In der Literatur beschriebene erhöhte Pb-B-Konzentrationen von Patienten, die zur Gerinnungshemmung mit Cumarin-Derivaten behandelt wurden (Handelspräparat: Marcumar^R) veranlaßten uns, den Pb-B-Spiegel von Marcumar-Patienten unter der Medikation zu messen. Die erhaltenen Meßwerte ließen eine wesentliche oder gerichtete Veränderung des Pb-B-Spiegels unter der Medikation nicht erkennen.

5. Patienten mit malignen Tumoren

In einer Studie an 15 Carcinom-Patienten wurde der Pb-B-Spiegel über einen längeren Zeitraum gemessen. Nahezu alle Blutbleiwerte lagen im Normbereich. Nur bei 2 Patienten mit ausgesprochener Kachexie lagen die Pb-B-Spiegel etwas über dem Durchschnitt. Im Verlauf zeigten die Blutblei-Konzentrationen dieser Patienten keine wesentlichen Veränderungen. Dabei ist besonders beachtenswert, daß auch die Personen mit schweren Knochenmetastasen keine wesentlichen Veränderungen des Pb-B-Spiegels aufweisen.

DISKUSSION UND SCHLUSSFOLGERUNGEN

1. Experimentelle Erhöhungen der Körpertemperatur über mehrere Tage führen bei beruflich nicht bleibelasteten Personen zu keinen Veränderungen des Blutbleispiegels.
2. Störungen des Säure-Basen-Haushaltes durch diätetische Umstellung auf extreme Kostformen mit alkalisierender und säuernder Wirkung über acht Tage rufen bei Normalpersonen und beruflich Blei-exponierten Personen keine Veränderungen des Blutbleispiegels und der Bleiausscheidung mit dem Urin hervor. Auch bei akuten respiratorischen und/oder metabolischen Acidosen sowie bei derartigen chronischen Zuständen im Rahmen von Schädel-Hirn-Traumata, Schlaganfällen und chronischer Niereninsuffizienz waren keine eindeutigen Veränderungen des Blutbleispiegels festzustellen.
3. Besondere physische und psychische Belastungen in Form eines Langstreckenmarches und Schnellfahrten bei der Deutschen Bundesbahn bewirken bei Normalpersonen ebenfalls keine erkennbare Bleimobilisation. Auch etwaige aufgrund hormoneller oder metabolischer Umstellungen in der Schwangerschaft diskutierte Veränderungen des Blutbleispiegels sind beim Vergleich mit nichtschwangeren Frauen desselben Lebensraumes nicht wahrscheinlich zu machen.
4. Erhöhungen des Blutbleispiegels aufgrund der Einnahme von Antikoagulantien vom Typ des Dicumarol (Marcumar^R) waren im Gegensatz zu den Befunden anderer Untersucher nicht zu sichern.
5. Auch Carcinome mit langem Krankheitsverlauf, z.T. schwerer Kachexie und z.T. ausgedehnten knochendestruierenden Metastasen führen anscheinend zu keiner besonderen Bleimobilisation.

Aus den durchgeführten Untersuchungen ist zu schließen, daß die als Folge verschiedener außergewöhnlicher Belastungen diskutierte Bleimobilisation aus den Knochen oder anderen Geweben - wenigstens bei erwachsenen Menschen ohne berufliche Bleibelastung - allenfalls gering ist und als Risikofaktor keine wesentliche Rolle spielt. Im Widerspruch zu dieser Annahme stehende Befunde oder Behauptungen anderer Autoren sind wahrscheinlich auf analytische Fehler zurückzuführen und bedürfen weiterer Klärung.

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Contractor: Bundesgesundheitsamt, Inst. für Wasser-, Boden- und Luft-Hygiene, D-1000 Berlin 33

Contract n° 091 - 74 - 10 ENV D

Project leader: Prof. Dr. K.AURAND, Prof. Dr. H.L.THRON

Title of Project: Study regarding the effects of heavy metals on man and the environment including epidemiological surveys (Nordenham field study).

For further elucidation of the quantitative relationship between chronic elevated lead exposure and parameters of biological effects in man comprehensive mass-screening of various population groups was performed near a large lead smelter (Nordenham/Weser) and in several control regions (Helgoland; Brake/Weser). At the same time atmospheric lead levels as well as particulate size-distribution were measured inside Nordenham city.

The study which had been preceded by limited surveys in Nordenham 1972/73, concentrated on

- the evaluation of exposure-dependent differences among population groups by different criteria,
- the biological sampling of risk groups among the population (pregnant women, children) for potentially high exposure,
- the examination of (mainly) Pb-exposed children to account for possible lead-associated disturbances of lung function and of peripheral nerve conductance.

Results:

1. Atmospheric concentrations of lead as obtained by quasi-continuous automatic monitoring of suspended atmospheric particulates during 1973 - 75 at 2.3 and 0.7 km distance from the smelter remained well below the standards defined 1975 by the E.C. Commission (Proposal for a Council Directive on air quality standards for lead): The annual mean levels were consistently below $1 \mu\text{g Pb/m}^3$; none of the calculated monthly averages exceeded $4 \mu\text{g Pb/m}^3$. Although there was a maximum daily mean level of $13.6 \mu\text{g Pb/m}^3$, only in 1974 more than 5% of all daily averages were found to exceed $4 \mu\text{g Pb/m}^3$. These comparatively low atmospheric lead levels were confirmed also in spring of 1976 when parallel measurements at 10 additional sites around the smelter were performed.

Repeat analysis of particle size distribution (Anderson non-viable impactor) resulted in a proportion of fine particles $< 5 \mu\text{m}$ effect. diameter of between 75 and 95 %.

2. Criteria of lead exposure

- Parameters:
- Lead content in peripheral blood (PbB) and in hair samples (PbH; children only),
 - δ -aminolevulinic acid dehydratase activity (ALA-D) in peripheral blood samples,
 - Free erythrocyte porphyrins (FEP) in peripheral blood samples.

Population groups sampled, number of subjects:

Regions	children		adults	puerperae/ neonates
	3-5 yrs	6-14 yrs	≥ 15 yrs	
Nordenham area	140	506	2850	379/365
Control areas (Helgoland, Brake)	30	235	2090	-

Results (PbB cf. also table!):

- Among adults, men were found to exhibit higher PbB levels than women. Sex-specific differences were less clear-cut for ALA-D and FEP.
- Occupational contact with lead had the greatest influence on criteria even as far as experience from epidemiological surveys in the general population is concerned because of its frequent persistence for many years after termination. Often family members of lead workers were affected to some degree as well.
- There was also a clear dependency of PbB on the distance between residence and the lead smelter for all groups. In adults, only minor influences on both ALA-D and FEP were found.
- In adults, non-specific influences were found to markedly affect both FEP (e.g. iron deficiency) and ALA-D (e.g. functional disorders of the liver, excessive alcohol consumption), thus invalidating usefulness of both parameters as criteria of lead exposure to some degree in this group.

3. Biological sampling of risk groups

Infants, Schoolchildren: There were no sex-specific differences among parameters measured in this group. For PbB and - frequently even more clear-cut - for ALA-D the influence of the distance between residence and the lead smelter was definitely more pronounced than in other groups; it was, however, evident for PbH and FEP as well. In children living closest to the smelter (cf. table: zone I), the requirements of the E.C. directive concerning PbB and ALA-D were not met.

Pregnant women, Neonates: Among these the lowest median PbB levels of all population groups studied were found (neonates < mothers < other adult females; cf. table), the latter difference being probably related to the lower hematocrit ratios in pregnancy. The influence of the distance from the lead smelter was evident as well, though less pronounced than in other groups.

Median FEP levels decreased in the following order: Neonates > puerperae > other adult females. These differences, however, could not be related to the individual degrees of Pb exposure. Definitely higher FEP levels than in other puerperae with similar PbB levels were found on the average in a group of pregnant women of mediterranean origin.

Studies on spontaneous mobilization of lead from body tissues

- Pregnancy: In 82 pregnant women with different degrees of lead exposure, there was no confirmed indication of lead mobilization from internal body deposits at childbirth.
- Physical exercise in adolescents: There was no definite indication of internal lead mobilization during or immediately after exhausting physical strain in adolescents (10-14 yrs) by a long-lasting sports event. An increased uptake of Pb-containing particulates may probably occur when performing e.g. football games on highly Pb-contaminated soil as is suggested by this study.

4. Examination of lung function and of peripheral nerve conductance

Lung function tests in children: Measurements of various lung function parameters were performed in each 56 male and female schoolchildren (8 - 10 yrs) living in Nordenham at various distances from the lead smelter. There was no definite association between lung function parameters and criteria of lead exposure.

Conduction velocity in peripheral motor nerves (N.ulnaris): In population groups comprising all ages (Pb-exposed persons: 288; controls: 692; mean PbB of groups: 10-22 $\mu\text{g}/100\text{ ml}$; maximum PbB: > 30 $\mu\text{g}/100\text{ ml}$), nerve conduction velocity in fast and slow motor fibres and distal latency time were measured in the distal segment of the ulnar nerve (SEPPÄLÄINEN & HERNBERG, 1972, modified). There was no definite association between changes in nerve function parameters and criteria of lead exposure.

Table: PbB distribution among various population groups as influenced by the distance between residence and lead smelter. Field survey in the Nordenham area (I-IV) and control regions (V)

Distance from smelter	I. 0 - 1	II. 1 - 2	III. 2 - 3	IV. 3 - 5	V. > 5 km
A) <u>Infants</u> , ♂, 3-5 yrs, Total N = 76	n : 14 \tilde{x} : 21.6 %>20: 71.4% %>30: 14.3% %>35: 0	27 16.9 33.3% 0 0	19 16.5 15.8% 5.3% 0	16 13.1 6.3% 0 0	
B) <u>Infants</u> , ♀, 3-5 yrs, Total N = 89	n : 21 \tilde{x} : 20.0 %>20: 47.6% %>30: 4.8% %>35: 4.8%	36 19.3 38.9% 5.6% 2.8%	15 16.3 0 0 0	17 12.7 0 0 0	
C) <u>Boys</u> , 6 - 12 yrs, Total N = 280	n : 32 \tilde{x} : 21.7 %>20: 62.5% %>30: 7.8% %>35: 6.2%	91 17.7 34.1% 1.7% 1.1%	29 13.5 0 0 0	43 12.6 0 0 0	85 11.9 0 0 0
D) <u>Girls</u> , 6 - 12 yrs, Total N = 271	n : 39 \tilde{x} : 22.1 %>20: 60.3% %>30: 17.9% %>35: 6.4%	78 18.2 37.8% 1.9% 0	24 13.8 16.7% 0 0	52 11.7 6.7% 1.9% 0	78 11.8 0 0 0
E) <u>Adults</u> , ♂, >15 yrs, Total N = 367	n : 9 \tilde{x} : 17.5 %>20: 33.3% %>30: 0 %>35: 0	90 15.1 17.2% 1.1% 1.1%	33 12.3 9.1% 3.0% 0	42 13.7 17.9% 7.1% 4.8%	193 13.2 8.6% 1.0% 1.0%
F) <u>Adults</u> , ♀, >15 yrs (except G), Total N = 425	n : 23 \tilde{x} : 13.0 %>20: 4.4% %>30: 0 %>35: 0	90 11.4 5.6% 3.3% 1.1%	62 10.7 0 0 0	54 9.3 1.8% 0 0	196 10.1 2.3% 0.5% 0
G) <u>Puer- perae</u> , Total N = 379	n : 26 \tilde{x} : 12.1 %>20: 9.6% %>30: 0 %>35: 0	112 10.1 2.7% 0 0	67 9.2 0 0 0	71 8.9 0 0 0	103 8.6 1.0% 1.0% 0
H) <u>Neo- nates</u> , Total N = 365	n : 23 \tilde{x} : 9.3 %>20: 10.9% %>30: 0 %>35: 0	109 9.0 1.8% 0 0	68 8.1 0 0 0	64 7.6 0 0 0	101 7.8 1.0% 1.0% 0

Comments: N, n = number of subjects

\tilde{x} = median of blood lead contents (PbB) expressed in $\mu\text{g Pb}/100\text{ ml}$

%>20/30/35 = percentage of PbB values > 20/> 30/> 35 $\mu\text{g}/100\text{ ml}$

Contractor: Prof. Dr. E. Küster

Contract n° 094-75-1 ENV. D

Project leader: Prof. Dr. E. Küster

Title of project: Effect of lead from immissions on the microbial activity in soil

Air polluted with lead derived from industrial immissions is dangerous for the growth of plants, and damage to higher plants has already been reported in many cases. Lead may also be assimilated from the soil through the root system. The different experimental records on the effect of lead from soil on the plant growth are related to solubility and availability of lead compounds in soil and sensitivity of plants against lead. The influence of lead on microorganisms has been examined on some bacteria and fungi with regard to specific physiological properties. Apart from a few studies about the effect of lead on the nitrification, very little is known about the effect of lead immissions on the microflora of the upper soil layers.

The behaviour and the effect of lead in soil is mainly determined by the properties of soil. Two soils with different contents of clay and humus were applied:

	<u>Clay</u>	<u>Organic Substance</u>	<u>Total Pb-content</u>
Soil A	17.30 %	1.7 %	20 ppm
Soil B	8.62 %	0.9 %	15 ppm

These soils were treated with various concentrations (100 - 5000 ppm) of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) or lead acetate ($\text{Pb}(\text{CH}_3\text{COO})_2$) resp. These lead compounds guarantee an homogenous distribution because of their solubility in water. This is necessary in order to avoid undesirable side-effects.

It has been shown that the figures of bacteria, actinomycetes, and fungi remain nearly constant at low Pb-concentrations, only decreasing after application of greater amounts of lead. The physiological groups examined are more affected by lead, even at low concentrations. The content of clay and humus is primarily responsible for the immobilization of lead in soil, and there exists an obvious relationship. A soil with a low absorption capacity exhibits a stronger lead effect on the soil microflora than one rich in clay and organic matter.

Besides the counting of microorganisms which does not sufficiently inform on the microbial activity, the following physiological criteria were examined:

CO₂-production
Dehydrogenase-activity
Nitrification

Soil respiration indicates the activity of the total soil microflora. It is reduced after the addition of 5000 ppm lead after 24 hours in soil A by 42.5 %, in soil B by 75.5 %. The CO₂-production of soil B is distinctly lowered after the application of smaller amounts of lead, as opposed to the clayey soil A. The examination of the dehydrogenase-activity confirmed the previous observation. This test is a further indicator for unspecific metabolic reactions of soil microorganisms. It was reduced in soil A by 47.1 %, in soil B by 80.2 %. Soil respiration and dehydrogenase-activity were drastically reduced by the addition of Pb-compounds. The grade of inhibition depends on the clay content of the respective soil.

The results of the examination of nitrification differ from those of the other microbial activities. The nitrate formation was slightly promoted in both the soil types by low concentrations of lead. Significant inhibition began at amounts of 2000 ppm. It increased after addition of 5000 ppm in soil A to 66.8 %, in soil B to 100 %. Ammonification and

nitrification are closely related. Ammonification, i.e. the activity of proteolytic microorganisms, is reduced by low Pb-concentrations. The nitrification was only reduced at higher Pb-concentrations. This may be explained by two different phenomena:

1. Inhibition of nitrification by a smaller amount of available ammonia because of a smaller number of proteolytic organisms reduced by lead.
2. Stimulation of nitrification by a greater amount of available ammonia set free from the clay by ion exchange.

It is concluded from these preliminary studies that a toxic effect of lead on the soil microflora can only generally be stated after consideration of all the single factors which contribute to a change of the microbial equilibrium in soil after lead addition.

Contractor : Université de Paris-Sud, Chatenay-Malabry

Contract n° 048-74-1 ENV F

Project Leader : Prof. Cl. Boudene

Title of project : Cycle du plomb dans l'environnement et ses rapports
avec son métabolisme chez l'homme

Four main aspects have been studied :

I. THE CONCENTRATION OF LEAD IN HUMAN BONES OF PARIS RESIDENTS

Lead was measured in a large number of human bone ash samples from the Paris area. Levels increased from birth up to 60 years of age. At birth levels were $5 \mu\text{g.g}^{-1}$; from 20 years onwards, they increased by $0.84 \mu\text{g.g}^{-1} \cdot \text{y}^{-1}$. At 60 years, they reached a plateau or a maximum value. Vertebrae were slightly more contaminated than ribs, and males more than females by 20 p. cent. Between 20 and 60 years, bone burdens increased by $5 \mu\text{g.d}^{-1}$ as an average. At 50 years bone burdens were 115 mg in males and 78 mg in females. A number of assumptions have been made to explain the stable levels observed after 60 years. The selection of less contaminated groups is among the most important and it justifies further investigations.

II. EXPERIMENTAL STUDY OF LEAD INHALED BY RATS

Distribution of inhaled lead is described in the rat. Animals were exposed for a short period to an aerosol which qualitatively and quantitatively simulates urban atmosphere. Exposure is followed by a solubilization of lead particles that agrees with the results reported after inhalation of soluble particles of lead. Clearance from the lungs is quite rapid. Some particles, deposited in the upper respiratory tract, migrate to the pharynx and are swallowed. Lead deposited in alveoli quickly appears in the blood. A part of the lead is excreted by the renal system, but the majority is transported to the skeleton, which is the main site of fixation of the metal.

III. MICROANALYSIS OF ^{204}Pb ISOTOPE

Improvements in the mass spectrometry of lead allowed some authors to work out most of the parameters of lead kinetics in the human body except precise behaviour of inhaled lead particles. This measure could be done after a single exposure of man provided aerosol is not toxic, and multiple blood samples accepted by volunteers.

Toxicity of a physicochemically well defined aerosol was tested in vivo in rats. No effects could be detected in lung till 100 $\mu\text{g}/\text{kg}$ body weight. Cytotoxicity on macrophages doesn't occur in vivo at this level after inhalation.

Detection of ^{204}Pb was thought to be possibly improved by ion probe microanalysis according to Castaing. Prior to mass spectrometry samples containing ^{204}Pb settled on silver sample holder (1 - 5 μl), foci (60 to 200 μm diameter) are extracted by accelerated oxygen ions. Signals are amplified by photomultiplier. Limit of the detection was found to be 0,02 10^{-5} μg by lyophilized μl . These results make possible inhalation and long term detection of ^{204}Pb in man (100 μg inhaled)

IV. LEAD IN MARINE ENVIRONMENT

Lead has been determined in sea for 9 months (12 samples per month) in the channel between Brest and Dunkerque, with a special attention to a reference area at La Hague.

Observations have been made on sea water, particulates, sediments and several aquatic species.

The quantitative estimation has been made by atomic absorption after preconcentration of the metal on colloidal manganese dioxide.

In the Seine Bay, water levels of lead are significantly higher than in the reference area. The pollution of marine species looks like low. Important amounts of metal are found in the small inferior fraction of the sediments, related to a beginning pollution in relatively unexpected areas.

LISTE DES PUBLICATIONS

C. BOUDENE, D. MALET, R. MASSE

Fate of ^{210}Pb inhaled by rats

Toxicology and Applied Pharmacology, 1977, 41, 271-276

C. BOUDENE, D. MALET, R. MASSE, J. GAUDIN

Generation of an aerosol comparable to fumes exhausted by motor vehicles.

Accepted by Atmospheric Environment

P. FRITSCH, R. MASSE, B. ARNOUX, J. CHRETIEN

Absence d'effets létaux du plomb inhalé, sur le macrophage alvéolaire in vivo

En préparation.

F. CINNOTI, G. RIBA, R. MASSE, C. BOUDENE

Dosage isotopique du plomb par microanalyse ionique

En préparation.

L. JEANMAIRE, F. PATTI, R. GROS, L. CAPPELINI, M. GARCET, J. LAPORTE

Teneur en plomb d'os humains provenant de la région parisienne.

C.E.A.-R. 4800, 1976, 26p.

P. GUEGUENIAT, P. BOVARD, J. ANSELLIN

Aspect du comportement du Plomb dans la Manche

A paraître

Contractant : Institut Pasteur de Lyon

N° du Contrat : 052-74-1 ENV F

Chef du Projet : MANUEL Yves, avec COLLE A., SIRE J., BOUCHERAT M.,
DEKNUOT Gh.

Titre du projet : Etude expérimentale chez le singe Rhésus du retentissement rénal de l'intoxication au plomb.

Introduction :

L'étude des protéinuries d'ouvriers fortement exposés au plomb a montré une augmentation de l'élimination urinaire de chaînes légères d'immunoglobulines, augmentation liée au taux de l'acide δ - amino levulinique urinaire. (1)

Or, l'augmentation des chaînes légères libres urinaires est considérée actuellement comme un début de mauvais fonctionnement du tube proximal rénal. (2)

Le présent travail se propose de vérifier expérimentalement chez le primate si, au cours d'une intoxication chronique au plomb, des singes d'un trouble de la fonction tubulaire rénale se manifestent. En outre il se propose d'étudier les variations de certains paramètres tant sériques qu'urinaires (Plombémie, Immunoglobulines, Aladéhydrase, copro et uroporphyrines.)

Enfin, en collaboration avec le laboratoire de génétique des mammifères du SCK-CEN de Mol (Belgique) un éventuel retentissement sur les chromosomes a été étudié sur les lymphocytes du sang périphérique de ces singes.

Protocole expérimental :

- Les intoxications ont été faites par voie orale sur 8 singes Macacus Irus par de l'acetate de Plomb incorporé à l'eau de boisson.

- 3 groupes de 2 singes ont reçu respectivement 1 , 4 et 10 mg de Plomb par 24h.

- 1 groupe de 2 singes a reçu 4mg par 24h en étant carencé en Calcium.

- 1 groupe de 2 singes, groupe témoin, n'a reçu que de l'acide acétique.

- 1 groupe de 3 singes a été intoxiqué au chromate de sodium pour induire une tubulopathie expérimentale à titre comparatif (injections sous-cutanées répétées de 10mg/Kg de chromate de sodium.)

Résultats :

1°) Evolution de la Plombémie :

Dès le 3^e mois la Plombémie s'élève au dessus de 400 μ g/l. Les taux sont maxima dès le 3^e mois ne variant que peu durant 16 mois. La Plombémie dépend de la dose ingérée, quoique non strictement proportionnelle.

Elle est la plus élevée chez les singes carencés en Ca⁺⁺.
Elle est le singe le plus précoce.

	Plomb mg/24h	3è mois	10è mois	16è mois	
Singe n° 1	0	160	170	120	Plombémie en µg/l
2	0	250	290	100	
4	1	470	520	650	
7	1	460	610	+	
3	4	560	430	+	
5	4	480	760	+	
10	10	780	880	+	
11	10	480	900	890	
carencés)2	4	1090	970	1130	
en Ca++(6	4	860	890	1340	

2°) ALA déshydrase : Les taux normaux sont 20 fois moins élevés que chez l'homme : l'appréciation d'une diminution est rendue aléatoire par la sensibilité de la technique : il semble que les singes ayant reçu la dose la plus faible (1mg/jour) aient seuls réagis.

3°) ALA urinaires : leur montée est très tardive et ne survient qu'au bout du 10è mois chez les singes 4 et 7 (1mg/jour) donc les plus faiblement intoxiqués (ils ont les taux d'ALA déshydrase les plus bas.)

4°) Les coproporphyrines urinaires : tous les singes intoxiqués ont une élévation significative : de 30 µg/l pour les témoins, elles atteignent 350 à 700 µg/l chez les singes intoxiqués dès le 10è mois, aucune élévation n'apparaît au 3è mois. Il n'y a par contre pas de corrélation entre l'importance de l'élévation des coproporphyrines urinaires et la quantité de Plomb ingérée.

L'uroporphyrine apparaît chez les seuls singes intoxiqués en moins de temps que s'élèvent les coproporphyrines, seulement au 10è mois.

5°) Les protéines sériques varient peu à l'exception des Immunoglobulines G qui diminuent chez les singes intoxiqués.

6°) Les aminoacides : les aminoacides sériques ne varient pas.

Par contre il semble que la valine et la 1 méthylhistine soient significativement augmentées dans les urines. Compte tenu des difficultés à évaluer la diurèse, ceci doit être vérifié.

7°) Les taux de Testostérone plasmatiques sont significativement différents chez les singes témoins et intoxiqués.

8°) Les protéines urinaires : les 10 singes montraient des profils électrophorétiques et immunoélectrophorétiques normaux avant l'intoxication. Après neuf mois d'intoxication, les singes 7 et 11 présentent une protéinurie très nette de type essentiellement glomérulaire, mais avec présence de fragments d'immunoglobulines et, également, une augmentation du taux d'excrétion de la Rétinol-Binding-Protein (surtout pour le singe n°7, qui en excrète 330 µg/24h.) Cette élévation est caractéristique des singes intoxiqués par le chromate de sodium.

Trois autres singes, malgré une protéinurie qui demeure normale, présentent des anomalies urinaires sous forme de " Minimal Change ".(2)

INTOXICATION PAR L'ACETATE DE PLOMB

Dose Plomb par jour mg	Protéinurie g/l		Electrophorèses Immuno-electroph.		Bêta-2 micro µg/24h		R.ð.P. µg/24h
	Avant	9 mois	Avant	9 mois	Avant	9 mois	9 mois
0	: 0,02	: 0,01	: N	: N	: 2,3	: 2	: 3
0	: 0,01	: 0,01	: N	: N	: 0,4	: 1,3	: 2
1	: 0,03	: 0,01	: N	: MCMixte	: 0,9	: 1,5	: 5
1	: 0,01	: 0,35	: N	: G	: 2,8	: 1,6	: 330
4	: 0,03	: 0,01	: N	: N	: 0,9	: 0,9	: 22
4	: 0,01	: 0,01	: N	: N	: 1	: 0,6	: 5
régime pauvre en Ca++	:	:	:	:	:	:	:
4	: 0,02	: 0,02	: N	: MC-Mixte	: 1,1	: 2,7	: 4
4	: 0,03	: 0,01	: N	: N	: 1	: 1,1	: 2
régime pauvre: en Ca ++	:	:	:	:	:	:	:
10	: 0,02	: 0,02	: N	: MC-Mixte	: 0,6	: 0,7	: 1
10	: 0,01	: 0,10	: MCG	: G	: 1,5	: 1,2	: 14

Type électrophoétique de la protéinurie :

N : Normal - G : Trouble de la filtration glomérulaire et de la réabsorption tubulaire. - MC : " Minimal Change "
Pro-téinurie quantitativement normale mais à profil qualitativement perturbé.

INTOXICATION PAR LE CHROMATE DE SODIUM

	$\beta_2 \mu$ (mg/l)	post- α (mg/l)	RBP (mg/l)
Singe I avant intoxication.	: 0,008	: 0,01	: 0,02
	diurèse de 130 ml		
1° "	: 16	: 53	: 96
	diurèse de 37 ml		
2° "	: 12	: 32	: 142
	diurèse de 18 ml		
3° "	: 6	: 2	: 20
	diurèse de 20 ml		

Les dosages ont été effectués 3 jours après les différentes injections de chromate de sodium, au moment où les protéinuries étaient maxima.

Le singe n°4 présente un trouble essentiellement glomérulaire.

Les singes 5 et 10 présentent des troubles mixtes tubulaires et glomérulaires (en particulier le singe n°5, qui excrète des quantités importantes de post- γ globuline, visible en électrophorèse en acrylamide-agarose).

Enfin, le singe n°2, malgré des profils électrophorétiques normaux, présente une excrétion légèrement augmentée de Rétinol-Binding-Protéin.

Il faut noter qu'il n'y a aucune augmentation de l'élimination de β -2 microglobuline, fait du au faible taux sérique de $\beta_2\mu$ chez le singe par rapport à l'homme. (3)

9°) Anomalies chromosomiques : dès le 10^e mois des anomalies chromosomiques apparaissent dans les lymphocytes de tous les singes intoxiqués (gaps, fragments), avec en plus des aberrations sévères chez les singes carencés en Ca^{++} . (4)

Conclusion :

Les signes rénaux sont faibles : la protéinurie est peu modifiée. Il y a une tendance nette à l'apparition d'un dysfonctionnement glomérulaire et/ou tubulaire, excepté chez les singes intoxiqués par le chromate de sodium.

Par contre 4 faits importants sont à retenir :

- l'importance de la Plombémie comme indicateur précoce d'exposition au plomb.
- les variations individuelles très importantes des réponses aux critères habituels (ALAurinaires, Coproporphyrines).
- le retentissement chromosomique, confirmé par une étude de faite sur des ouvriers exposés au plomb. (5)
- le rôle important du Ca^{++} .

O'autres points seront précisés par une étude ultérieure :

- une cinétique précise de la Plombémie.
- l'évolution des anomalies après arrêt de l'intoxication.
- le comportement des Immunoglobulines.
- les anomalies des aminoacides urinaires.
- une possible influence sur la Testostérone.

Summary :

10 Cynomolgus monkeys (Macacus Irus) were given 0,1,4 or 10 mg of lead 6 days a week for 16 months. 2 monkeys also receiving 4 mg were kept on a low Calcium diet. The main results were :

- Blood lead concentration increases rapidly under treatment depending on the dose of lead and was enhanced by a low calcium diet.
- Increasing of urinary amino-levulinic acid and coproporphyrins varies with each individual and appears late.
- Lead treatment increases significantly chromosome abnormalities especially when it is associated with a low calcium diet.
- Proteinuria remains low except for 2 monkeys. A significant tendency to a light glomerular and/or tubular defect is noted.

Some others slight modifications are discussed,

Références :

- 1 COLLE A., CHAMBON P., MANUEL Y.
Protéinurie après exposition au plomb. Aspects expérimentaux. Actes du Congrès " Rein et Toxiques" LYON 1974 MASSON Ed. p. 283-289.
- 2 MANUEL Y., GREELAND T.B.
Immuno-electrophoresis : a convenient method of studying "minimal change" in proteinuria.
In : Protides of the biological fluids, Proc. of the 21th colloquium Bruges, 1973, Elsevier AMSTERDAM p. 393-400.
- 3 COLLE A., SIRE J., JEANNIN M., MANUEL Y.
Néphropathie Expérimentale et β 2 microglobuline chez le singe. In colloque Européen sur la β 2 microglobuline. Lyon. Novembre 1977.
- 4 DEKNUOT Gh., COLLE A., GERBER GB.
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Mut. Res. 45 77-83 (1977)
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Chromosomal aberrations in workers professionally exposed to lead.
En cours de publication.

Contractor : ENI - SNAMPROGETTI, San Donato Milanese (Mi)

Contract n° : 059-74-7 ENV I

Project leader : P. Garibaldi

Title of project : The use of isotopically differentiated lead in petrol additives

This research project is aimed at assessing the contribution of lead emitted from motor vehicle exhaust to environmental contamination and to the human body burden of lead.

Antiknock compound with isotopically differentiated lead is used as tracer.

During the period September 1974-December 1975, the phase zero has been carried out.

Negotiations for the supply of isotopically differentiated lead (206/207 = 1.04) from Broken Hill, Australia have been completed and the SIAC Company, which supplies about 80% of the Italian market, has switched since August 1975 all its production to this lead.

Environmental and blood samples have been taken to assess the background level of lead concentrations and the isotopic ratios. Up to the end of 1975 a total of 2800 samples were collected and analysed. The results obtained gave for the isotopic ratio 207/208 a value of 1.18 ± 0.03 .

At present the province under study, Piedmont in Northern Italy, is fully supplied with isotopically differentiated tetraethyl lead. The analyses* carried out within the framework of a detailed sampling programme of petrol from the petrol stations have confirmed this.

Other experiments designed for this research are in progress in order to provide answers to these specific points :

- 1) Determination of the contribution of automotive traffic to atmospheric lead pollution in selected urban and rural areas.
- 2) Identification of critical transfer pathways of automotive lead to man.
- 3) Evaluation of the contribution of automotive lead to absorption in humans (including children) through inhalation and ingestion.
- 4) Estimation of the distribution of automotive lead into the various compartments of the ecosystem.

* in collaboration with the Joint Research Centre at Ispra.

Contractor: University of Amsterdam, Coronel Laboratory

Contract No. 093-75-1-ENV N

Project leader: Prof.Dr R.L.Zielhuis

Title of project: Sexual impact on susceptibility of haemsynthesis to inorganic lead

The project was designed to explore possible determining factors associated with female susceptibility to inorganic lead, as regards haemsynthesis. In this study particularly the relation between lead in blood (PbB), serum iron (Fe-S) and free erythrocyte protoporphyrin (FEP) levels in non-occupationally exposed male and female subjects were studied. In addition the possible effect of oral contraceptives (hormones) on above mentioned parameters in females was studied.

In April/May 1976 students of the Medical Faculty, University of Amsterdam, 19-21 yr of age, were studied: 57 males (group MI), 47 females without use of oral contraceptives (group FNC) and 44 females taking oral contraceptives (group FC). Blood was taken between 9.00 and 10.30 am, and examined blind. In addition to PbB, Fe-S and FEP also haemoglobin (Hb), haematocrite (Ht), total-iron-binding-capacity (TIBC) and unsaturated-iron-binding capacity (UIBC) were measured. The oral contraceptives most used were: mycrogynon (34%), neogynon (16%), stederyl (11%) and orthonovum (11%). In November-December 1976 another group of 51 male students of the same age (group MII) was examined; PbB and FeS levels were measured two times at 9.00-10.00 am and at 3.00-4.00 pm; FEP was not measured.

The data from groups MI, FNC and FC were as follows:

	MI (n=57)	FNC (n=45-47)	FC (n=43-44)
PbB (ppb)	138 \pm 39	130 \pm 42	143 \pm 38
FEP (μ g/100 ml rbc)	33.1(25.1-43.7)	37.2(26.3-52.5)	36.3(26.9-49.0)
Hb (mmol/l)	10.0 \pm 0.5	8.9 \pm 0.6	8.9 \pm 0.5
Ht (%)	45.4 \pm 2.4	41.2 \pm 2.3	40.9 \pm 2.3
FeS (μ mol/l)	22.1 \pm 7.8	18.4 \pm 6.5	24.0 \pm 7.3
TIBC (μ mol/l)	62.8 \pm 6.4	63.6 \pm 9.8	72.6 \pm 11.3
UIBC (%)	64.7 \pm 12.3	71.0 \pm 9.7	66.4 \pm 10.5

(arithmetic averages \pm s.d., except for FEP: geom.av. + range)

The following significant differences were found:

MI - FNC: FEP ($P < 0.05$), Hb ($P < 0.0005$), Ht ($P < 0.0005$), Fe-S ($P < 0.01$), UIBC ($P < 0.005$)

FNC - FC: Fe-S ($P < 0.0005$), TIBC ($P < 0.0005$), UIBC ($P < 0.025$).

The linear relationships for PbB x Fe-S were as follows:

MI, $r = -0.36$, $P < 0.01$; FNC, $r = +0.21$, $P > 0.05$; FC, $r = +0.18$, $P > 0.05$;
FNC+FNC, $r = +0.25$, $P < 0.05$.

In MII the relationship between PbB and Fe-S showed a negative trend: $r = -0.14$, $P > 0.05$; pooling of MI and MII: significant relationship $r = -0.28$, $P < 0.01$.

In MII there was no difference between Fe-S levels in morning and in afternoon.

The results of this study of non-occupationally exposed males and females can be summarized as follows:

- FEP levels in females are higher than in males at same PbB-levels; there is no effect of oral contraceptives on FEP
- Fe-S levels in females, not using oral contraceptives, are lower than in males, but in females using oral contraceptives Fe-S levels are about the same as in males; the unsaturated iron binding capacity in males is lower than in females, particularly in those not using oral contraceptives; use of contraceptives increases the total iron binding capacity
- in males there is a trend for decreasing Fe-S levels with increasing PbB-levels, whereas in females the trend is just the opposite.

The study did not observe any effect of type of oral contraceptive, neither of last date of menstruation on Fe-S levels.

This study casts doubt on the hypothesis that the difference in FEP-levels in males and females could mainly be due to a difference in iron-body stores, because in females using oral contraceptives there was no difference in FEP-level but very much in Fe-S level in comparison to females not using these. However, Fe-S levels may not be very good indicators for the metabolic availability of iron, so the hypothesis cannot be rejected either. The reported difference in the trend between PbB- and Fe-S levels in males and females needs to be confirmed before being accepted as generally true. The apparent difference in FEP level and in susceptibility of haem synthesis to inorganic lead may be due to difference in endocrinological status. This should be explored in animal experiments and in epidemiological studies; one could examine whether such a difference starts to appear in pubescents-adolescents, moderately exposed to lead.

This study has been published as follows:

A.A.E.Wibowo, P.del Castilho, R.F.M.Herber, R.L.Zielhuis, Blood lead and serum iron levels in non-occupationally exposed males and females, *Int.Arch.occup. environm.Hlth*, 39, 113-120 (1977).

Contractant : Unité de Toxicologie Industrielle et Médicale U.C.L.

N° du contrat : 035-74-1 ENV B

Chef du projet : Professeur R. Lauwerys

Titre du projet : Lead toxicity : Human and Animal Studies.

I. HUMAN INVESTIGATIONS.

I.1. Response of some heme biosynthetic pathway parameters in men, women and children moderately exposed to lead +

The responses of various indices of the heme biosynthetic pathway [δ-aminolevulinate dehydratase (ALAD) in erythrocytes, free erythrocyte porphyrin (FEP), δ-aminolevulinate in urine (ALA-U)] to a moderate lead-exposure (blood-lead concentration, Pb-B = 15 to 45 µg/100 ml blood) have been compared between groups of adult male lead-workers, adult non-pregnant female lead workers, and school-age children (11 years old) living in areas differently polluted by lead (rural and lead smelter area). Control groups (non smokers) of adult men and women were also examined. The groups of Pb-exposed men and women showed an identical mean value for Pb-B, i.e. 29 µg/100 ml, indicating a similar degree of exposure to lead for both groups. Compared to the control groups, the women exhibited a significant increase for FEP and ALA-U, whereas men only for FEP. Significant correlations have been found between Pb-B and log FEP for women ($r = 0.74$) and men ($r = 0.54$) but for the same Pb-B increment women exhibit a larger FEP increase than men. ALA-U does not significantly

Part of this work has been performed in collaboration with Drs. Bruaux P., Claeys-Thoreau F., Van Overschelde J. and Professor A. Lafontaine (Institut d' Hygiène et d' Epidémiologie, Ministère de la Santé Publique, Brussels, Directeur Professeur A. Lafontaine).

The follow-up of the children in the lead smelter area and in the rural area is pursued in collaboration with the Institut d' Hygiène et d' Epidémiologie and is presently sponsored by the Commission Interministérielle de la Programmation Scientifique (CIPS).

change in men when Pb-B does not exceed 40 - 45 $\mu\text{g}/100\text{ ml}$, but in women a significant correlation ($r = 0.47$) between ALA-U and Pb-B is already found when Pb-B increases from 15 to 45 $\mu\text{g}/100\text{ ml}$. Compared with the rural children (Pb-B, 9.4 $\mu\text{g}/100\text{ ml}$, FEP 64 $\mu\text{g}/100\text{ ml}$ erythrocytes, ALAD 54 units) the children living at less than 1 km from a lead-smelter exhibited a significant increase of Pb-B (30 $\mu\text{g}/100\text{ ml}$) and FEP (137 $\mu\text{g}/100\text{ ml}$), an inhibition of ALAD (26 units), a slight positive correlation of ALA-U with Pb-B, but no biological signs of anaemia. In children living at approximately 1.5 km from the plant, there is still a significant increase in Pb-B (21 $\mu\text{g}/100\text{ ml}$) and a concomitant inhibition of ALAD (38 units), but no change in FEP concentration. Comparison of the dose response curves between Pb-B and FEP in men, women and children indicates that the sensitivity to lead is in the order of children \gg women $>$ men. Significantly increased FEP ($>$ mean FEP value of control group + 2 SD. = 82 μg per 100 ml erythrocytes) in children, following long-term low level lead exposure should be regarded as an early biochemical sign of undue lead absorption and provides a reliable basis for the proposal of 25 μg Pb per 100 ml as the maximum allowable concentration of lead in blood of school-age children.

I.2. Evaluation of the transplacental transfer of lead in pregnant women (and its possible biological effects) and study of the influence of the place of residence on exposure to lead during foetal life ⁺

In 503 pregnant women living in different areas of Belgium (rural, semi-rural, urban) blood was withdrawn at delivery. Cord blood and a fragment of total placenta was also collected. A questionnaire was applied to each mother to get informations mainly on

This work is part of a research project sponsored by the Belgian Ministry of Health in which the placental transfer of two other heavy metals (mercury and cadmium) is also investigated. An extension of this survey to pregnant women living in an area (Libramont) where the drinking water is possibly contaminated by heavy metals (in particular lead) is presently undertaken under the sponsorship of the Commission Interministérielle de la Programmation Scientifique (CIPS).

their successive residences since birth, occupation, smoking and drinking habits and husband's occupation. The frequency distribution of Pb-B in mother ($< 20 \mu\text{g}/100 \text{ ml} : 98 \% ; < 30 \mu\text{g}/100 \text{ ml} : 99.9 \% ; < 35 \mu\text{g}/100 \text{ ml} : 100 \%$) is well below the maximum allowable distribution proposed by CEC. The distribution of Pb-B in the newborns parallels that found in the mothers with a slight shift to the left (about $2 \mu\text{g}/100 \text{ ml}$ at each frequency). There is an excellent correlation between Pb-B mother and newborn ($r = + 0.808$). For the range of Pb-B observed in the mothers and their newborns there is no significant correlation between Pb-B and FEP. On the contrary ALAD is negatively correlated with Pb-B in mother and newborn. The correlation is higher when ALAD activity is expressed in per cent of its activity found in the presence of the reactivator dithiothreitol rather than in absolute values. The median value of Pb-B in placenta was $7.5 \mu\text{g}/100 \text{ g}$ (wet weight). A slight but statistically significant effect of environmental pollution by lead (urban and industrial $>$ semi-rural $>$ rural area) on lead uptake by the pregnant mothers and its transfer to their foetus was demonstrated. However this slight difference in lead exposure had no significant influence on the biological parameters determined during this study (FEP, ALAD, Hb, Htc).

II. ANIMAL INVESTIGATIONS

II.1. Evaluation of the effect of lead administered orally on some parameters of the heme biosynthetic pathway in various tissues of adult male and female rats.

Lead was administered to male and female rats in drinking water for 3 and 6 weeks at the following doses : 0, 10, 100, 1000, 5000 ppm and for 6 months at 10 ppm only. Various blood (Pb-B, Htc, Hb, FEP, ALAD, reticulocytes) and tissue (ALAD, Free tissue porphyrins, Pb-T) parameters were determined. Pb-B increases with dose but reaches rapidly a plateau despite continuous Pb-administration. Concentration of Pb in kidney, liver and brain correlates with Pb-B. Pb does not accumulate in heart. Kidney is the main site of Pb deposition and kidney ALAD is the parameter most susceptible

to lead, since reduction is observed in all treated groups after 3 weeks of exposure. However kidney ALAD inhibition is transitory since after 6 weeks it is only observed in the 5000 ppm group. At 10 ppm lead prevents also the increase in blood ALAD activity normally associated with the reticulocytosis of repetitive bleeding. The next parameters affected by lead are : ALAD in blood which is inhibited after 6 weeks of treatment with 10 ppm lead, and FEP, ALA-U, and FTP in kidney which are increased after 3 or 6 weeks of treatment with 1000 and 5000 ppm lead. On the basis of the parameters selected and the period of observation (up to 6 months) it appears that 10 ppm is close to the no-effect level in adult rats. This dose and lower ones were therefore selected for administration to pregnant animals.

II.2. Evaluation of the no-effect level of lead on some parameters of the heme biosynthetic pathway in various tissues of newborn rats following short-term or long-term oral administration of lead to the mothers

II.2.1. Effect of short-term administration of lead to pregnant rats

- Lead was administered to adult female rats in drinking water (0 ; 0.1 ; 1 and 10 ppm) for 3 weeks before mating, during pregnancy and during three weeks after delivery. On day 21, after delivery the mothers and their newborns were sacrificed.
- In mothers a significant increase of Pb-B and Pb concentration in kidney was found in the 10 ppm group, but this increase in lead concentration was not associated with any statistically significant modification of the biochemical parameters.
- In newborns, lead concentration in blood and in kidney was also significantly increased in the 10 ppm group and this lead exposure was associated with a decrease of the ALAD activity in blood and an increase of FTP in kidney.
- On the basis of the biochemical parameters investigated one can therefore conclude that the developing organism is more susceptible to the biological action of lead than the organism of adult animals and that the "no-effect" level of lead administered

during pregnancy and in the neonatal period is around 1 ppm.

II.2.2. Effect of long-term administration of lead to pregnant rats

Lead was administered to three groups of 4 weeks-old female rats at the dose of 1 ppm in drinking water. The first group received lead continuously for 150 days before mating (with non-lead-treated male rats), during pregnancy and during 3 weeks after delivery. In the second group lead was also administered for 150 days before mating but was withdrawn during pregnancy and during the post natal period. In the third group lead was administered for 150 days : the animals were then kept without lead exposure during the 50 days before making them pregnant, during pregnancy and during the post natal period. A control group received only demineralized water. On day 21 after delivery the mothers and their newborns were sacrificed. In mothers and in newborns lead concentrations in blood and in soft tissues were increased over control values only in the group in which lead had been stopped for 50 days before pregnancy. This lead accumulation in soft tissues was associated with an increased FIP in liver, heart and brain of the mothers and in liver of the newborns. This observation suggests that lead stored in the organism can be mobilized during pregnancy. If such a phenomenon occurs also in woman, not only pregnant women should be removed from lead exposure but also any woman during her reproductive life.

II.2.3. Effect of lead on lactating rats and their sucklings

- Lead was administered to lactating rats in drinking water (0;1; 10 and 100 ppm) from the day of delivery up to day 21 at which time the mothers and their newborns were sacrificed.
- In mothers, a significant increase of Pb-B and a reduction in ALAD activity of blood were found in the 100 ppm group. In tissues Pb was significantly increased in liver of the 100 ppm group and in kidney of the 10 and 100 ppm groups. None of the biochemical parameters measured in tissues was significantly modified.

- In suckling rats an increase in Pb-B and a reduction of ALAD activity in blood were found in the 10 and 100 ppm lead groups. Pb concentration was significantly increased in liver, kidney and brain of the 100 ppm group and in kidney of the 10 ppm group. Lead storage in kidney of the 100 ppm group was associated with a marked increase in FTP and a slight reduction in ALAD activity.
- On the basis of the biochemical parameters studied in the newborn rats, the "no-effect" level of lead administered in drinking water during lactation is around 1 ppm, which is rather similar to that found when lead was administered to the mother before and/or during pregnancy.

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2. ROELS H.A., BUCHET J.P., LAUWERYS R., HUBERMONT G., BRUAUX P., CLAEYS-THOREAU F., LAFONTAINE A. and VAN OVERSCHELDE J., Impact of air pollution by lead on the haem biosynthetic pathway in school-age children. Arch. Environ. Hlth. 31, 310, 1976.
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5. BUCHET J.P., LAUWERYS R., ROELS H. and HUBERMONT G., Mobilization of lead during pregnancy in rats. Int. Arch. Occup. Environ. Hlth 1977 (under press).
6. ROELS H., LAUWERYS R., BUCHET J.P. and HUBERMONT G., Effect of lead on lactating rats and their sucklings. Toxicology (under press).
7. LAUWERYS R., BUCHET J.P., ROELS H., HUBERMONT G., Placental transfer of lead, mercury, cadmium and carbon monoxide in women. I. Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. Environ. Research (under press).

8. BUCHET J.P., ROELS H., HUBERMONT G., LAUWERYS R.,
Placental transfer of lead, mercury, cadmium and carbon monoxide in women. II. Influence of some epidemiological factors on the frequency distribution of the biological indices in maternal and umbilical cord blood. Environ. Research (under press).
9. ROELS H., HUBERMONT G., BUCHET J.P., LAUWERYS R.,
Placental transfer of lead mercury, cadmium and carbon monoxide in women. III. Factors influencing the accumulation of heavy metals in placenta and relationship between metal concentration in placenta and in maternal and cord blood.
Environ. Research (under press).
10. ROELS H., BUCHET J.P., BERNARD A., HUBERMONT G., LAUWERYS R.,
MASSON P., Investigations on factors influencing exposure and response to lead, mercury or cadmium in man and in animals.
Environ. Health Perspectives (under press).

Contractant : Belgian Nuclear Centre S.C.K./C.E.N. - Mol (Belgium)

N° du contrat : 036-74-7 ENV.B

Chef du Projet : Prof. O. VANDERBORGH

HUYBREGHTS Gerrit, VAN PUymbROECK Sylvain

Titre du Projet : Mobilisation of ^{210}Pb from skeleton in mice

Introduction

Lead, introduced in the body, concentrates in the bones (e.g. Lloyd R.D. et al., Health Physics 28, 575-583, 1975), where it is assumed to be withdrawn from the toxicologically active pool of "soft tissue lead". A few authors mention that lead could possibly be mobilised from these bone stores by different physiological stresses, such as infections and acidosis (Bruaux P., CCE Semin. Metab. Plomb, Luxemb. 25 avril 1972, P. III-17) ; such a mobilisation could induce an increase of the concentration of Pb in the soft tissues and thus enhance the toxicity of the formerly inactive Pb-store. However, we could not find any quantitative experimental evidence in the literature to confirm these assumptions. Barry P. (C.E.C. Symp. Environmtl. Health Aspects of Lead, Amsterdam 1972, p. 415-426) rejects the possibility and states that the mobilised quantities are too small to produce a significant rise of the Pb-concentrations.

The present experiments were conducted in order

1. to compare the effect of different treatments (hormonal, chelating agents, diets) on the mobilisation of Pb ; and especially the effect of alginate (a diet additive forming gels with water), which has proven to affect the mobilisation of assimilated ^{85}Sr (Vanderborgh O. et al., CONF-720818, p. 397-401, 1973), while it affects only moderately the assimilation of Pb in rats on a milk diet (Carr T., Nature 224, p. 1115);
2. to compare the effect on mobilisation, of different latency periods between the ^{210}Pb -injections and the start of the mobilising treatments;
3. to trace the effect of the age of the animals at ^{210}Pb -injection ; it is indeed known that younger organisms are more vulnerable to Pb-intoxication (Chisolm J., Develop. Med. Child Neurol. 7, p. 529-536, 1965).

Summary of the experimental treatments

Mice received a ^{210}Pb -injection at 1 or 3 months of age. After injection, a resting period of 4, 18 or 34 days allowed the injected Pb to distribute through the animals, during the resting period the mice were kept on their standard pellets. After this latency period, the mobilising treatments were started. To obtain an enhancement of the ^{210}Pb -concentration in the blood, 5 such treatments were applied during six days (controls receiving 2 x per day I.P. glucose + stand. pellets);

1. 5 % alginate was mixed in a dough (flour, water, milk), and was given at libitum as the only food ; water was available at libitum in all experimental groups ;
2. parathyroid hormone extract was given by subcutaneous injection 3 times per day (= 10 U.S.P. parathyroid units per day) ; + standard pellets ;
3. Na_3CaDTPA (diethylenetriaminepenta-acetic acid) was given in one daily dose of 0.025 mmol per animal (about = 1 mmol.kg^{-1}) ; + standard pellets;
4. Intraperitoneal injection of 0.1 ml of a 1 % sodium alginate in demineralised water twice per day ; + standard pellets ;
5. Combined treatment, in which the alginate diet was given together with the DTPA injection and the alginate injection.

Urinary and faecal excretion of the animals was daily and individually and separately collected, ^{210}Pb -content of the blood of each animal was measured at the end of the experiment, as well as the ^{210}Pb in femur, kidney, liver, spleen, muscle sample and in the whole body. In total, 30 experimental groups of six mice each were obtained (2 age groups times 3 Pb-age groups times six treatment groups) ; the group that was 3 months old at Pb-injection and that underwent a latency period of 4 days between Pb-injection and mobilising treatment, was not included in the results. The number of samples analysed for ^{210}Pb increased to about 5000, with the preliminary experiments included.

Summary of the results

The treatment with the alginate containing diet induces a considerable increase in the concentration of ^{210}Pb in the blood of the mice ; table I summarises the results. Thus, the alginate-diet group which was 1 month old when injected with ^{210}Pb and which waited 34 days thereafter to begin with the mobilising treatment, had 86 % more ^{210}Pb in the blood, compared to the equivalent control group. In this same group of animals (1 month old at

injection with ^{210}Pb) we can also observe that the mobilising effect is more pronounced for the older Pb-concentrations : indeed the 34 resp. 18 and 4 days latency period corresponds to an increase in ^{210}Pb of 86, resp. 51 and 19 %, compared to the control groups on the standard diet.

Table I : ^{210}Pb in the blood of mice at the end of a six-days treatment period (arbitrary units, corrected for the body-burden of each animal)

Age of Pb-contamination at the start of the mobilising treatment		→ 34 days		18 days		4 days	
Age of animals when they got the ^{210}Pb -injection:	Mobilising treatment groups	(\bar{x})	s_x	(\bar{x})	s_x	(\bar{x})	s_x
1 month	A. Controls	1.36	0.11	2.71	0.40	9.82	1.23
	B. Algin diet	2.53***	0.19	4.09***	0.86	11.64***	0.67
	C. PTHormone	2.03***	0.21	3.39***	0.46	10.74(4)	1.34
	D. Algin I.P.	1.24	0.18	1.93**	0.38	8.75	0.82
	E. DTPA	0.84***	0.11	1.23***	0.20	7.70***	0.77
	F. Combined	1.42	0.38	2.16	0.66	9.35	2.14
Age of mice at ^{210}Pb -injection:	A. Controls	1.54	0.50	2.87	0.76		
	B. Algin diet	1.85	0.62	4.85***	0.33		
	C. PTHormone	1.74	0.39	3.21	0.58		
	D. Algin I.P.	1.24	0.81	3.03	0.77		
	E. DTPA	0.80***	0.25	1.88***	0.31		
	F. Combined	1.67(4)	0.10	2.85(4)	0.66		

*** Differences with the control groups significant at the 5 % levels
 **: Differences with the control groups significant at the 1 % levels

The urinary output of ^{210}Pb is also increased (maximally 60 %) ; the same holds to a lesser extent for the faecal excretion in some groups (maximally 27 %) treated with algin-containing dough. If alginate diet is by far the most effective to increase the ^{210}Pb -level of the blood, it is DTPA that increases up to 6 times the urinary excretion of this Pb, without any significant influence on its faecal excretion.

As well the liver as the kidney show a 200 to 300 % increase of their ^{210}Pb -content in the groups maintained on algin-dough.

The younger animals did not show to be more affected by the mobilising treatments. Eventually, the results show an antagonistic effect of the alginate containing diet on the DTPA-effects, and this could be an indication that alginate has to be avoided when the DTPA treatment is of the utmost importance, and vice-versa. This antagonism is illustrated by the fact that the DTPA-treated groups show a sharp decrease in ^{210}Pb -concentration in the blood, this effect is reversed to an increase in the groups receiving the combined treatment of DTPA + alginate diet.

Liste des publications

G. HUYBREGHTS, O. VANDERBORGHT, S. VAN PUymbROECK
Mobilisation of ^{210}Pb and stable lead in mice, effects of the age of animals and of contamination (in preparation).

Contractor : E.C. Environmental Research

Contract n° : O37-74-7 ENV.B

Project leader : A. LEONARD and G.B. GERBER

Title of project : Studies into the toxic action of lead in biochemistry
of the developing brain and on cytogenetics of post
meiotic germ cells.

Our work was concerned with different aspects of lead toxicity :

- 1) Intestinal absorption of lead in the rat.
- 2) Effect of lead on early postnatal development of the brain in the rat.
- 3) Effects of lead on fertility and embryonic development in the mouse.

1. Intestinal absorption of lead in the rat.

Absorption of dietary lead by intestine represents the most important source of body lead. We are studying different factors influencing lead absorption, using an intestinal in vivo preparation.

In our procedure, radioactive lead is injected into the intestinal lumen and all blood flowing through the portal vein is collected while simultaneously blood loss is replaced by an intravenous infusion. The data show that immediate absorption is about 3% of the dose injected per min. and that this value remains independent of the amount of lead given, confirming that active transport does not play a role. Deprivation of Ca decreases rather than increases lead absorption, and young animals absorb about 2-3 times more lead than adult ones.

Absorption is higher in the jejunal part than in the duodenum or colon.

2. Effect of lead on early postnatal development of the brain in the rat.

We have developed a method to measure regional blood flow in different structures of the rat brain. Microspheres (15 μ) marked with ^{144}Ce are injected in the left heart ventricle and are captured in the capillaries. The brain is then sectioned and stained for myeline, radioautographs are prepared, and the microspheres in the different structures are counted. In control animals, blood flow is higher in the cortex and lower in the white matter, while other structures show intermediary

values. In rats fed with 1% of lead in diet from birth, blood flow is not diminished in the grey matter, showing that saturnian encephalopathy is not due to a deprived blood flow. On the other hand, inferior cerebral structures and cerebellum show diminished values of blood flow compared to the controls.

3. Effects of lead on fertility and embryonic development in the mouse.

Female mice were mated and given a diet containing 0, .125, .250, .500 or 1.00% of lead from different times of the gestation. They were dissected on days 16, 17 or 18 of gestation and the presence, number and weight of embryos were determined.

- Doses of lead above .125% administered from day 1 reduce significantly the incidence of pregnancies (mothers with at least 1 implant at dissection). Among the pregnant females, the preimplantation loss is not greater after lead treatment, but the postimplantation loss increases and the weight of the embryos diminishes.
- When given at the beginning or at the end of implantation (day 6th or 7th), doses of .500% induce abortion in 90 or 80% of the animals.
- Doses of 1.00% of lead given at the end of the principal organogenesis period (day 10th to day 13th) induce abortion in all cases.

Different experiments were undertaken to study the mechanisms of the inhibition of the implantation by lead.

- A potential effect of lead on the chromosomes of somatic cells might explain the inhibition of the implantation, or the increase of fetal death. Mutagenicity of lead has been extensively studied, but the results obtained so far have been ambiguous and inconclusive. Young female mice were therefore treated with different doses of lead (0, .250, .500 and 1.00%) in diet for periods up to 3 months. Chromosomes of the bone marrow cells were then examined for the presence of abnormalities. In addition, another test for mutagenic action, the number of micronuclei, was carried out.

No severe chromosome or chromatid aberrations were observed at any dose level and the number of micronuclei in polychromatic erythrocytes remained at control levels after lead treatment. Although studies on embryonic chromosomes must confirm our conclusions, embryonic or fetal death seems thus not due to chromosomal factors.

- The early development of the embryos was studied by counting the number of cells in embryos 48 hours after successful mating and treatment with the different doses of lead. Lead was found to cause a variable delay of the first cell division and this effect is increased with the dose given.
- The development of the embryo until day 7 (day of presumed implantation) and the reaction of the mother were studied in histological sections of the uterus, oviduct and ovary from normal and lead-treated mice (.500% in diet). Lead treatment does not prevent blastocyst formation, although blastocysts tend to remain smaller. Differentiation of giant cells in the trophoblast and of decidual cells in the uterus is, however, impaired, and the corpora lutea remain underdeveloped. The analysis of the different factors influencing implantation of the normal embryo indicates that the impairment of implantation is mainly due to a lack of progesterone. This is suggested by the aspect of the corpora lutea and by preliminary results with radioimmunoassays of the hormones.

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Contracting party : UNIVERSITE LIBRE DE BRUXELLES

Service de Géologie et Géochimie Nucléaires,
Faculté des Sciences.

Number of contract : 039-74-1 ENVB

Project Director : E. PICCIOTTO

(Senior scientist : D. PETIT)

Title of project : Investigation on the origin of natural or industrial lead in the environment in Belgium and neighbouring countries, based on its isotopic composition.

Due to the ubiquity of lead in the environment, it is often difficult to estimate the contribution of lead pollution sources in the geochemical and biological cycles.

As opposed to other heavy elements, variation of lead isotopic composition may be significant, depending on its geological origin. Stable lead is composed of the following isotopes : Pb-204, Pb-206, Pb-207 and Pb-208. The last three are the final stable products of the three natural radioactive families and are called, for this reason, radiogenic. The lead ratios are therefore a function of the parent uranium-thorium abundances, their decay rates and the system age. Older is an ore, less abundant are the radiogenic isotopes (206, 207 and 208) in comparison with isotope 204.

Since about ten years, works carried off in the United States, based on this property, considered the possibility to identify the principal source of lead pollution in the environment, namely the lead alkyls used as antiknock additives for automobile gasoline (1). In most situations, the isotopic composition of lead added in the gasoline of this country varied with time and moreover was shown to be not significantly different from that of other anthropogenic sources and from natural sources (for a critical survey of the published papers, see 2).

element. A large increase of lead concentration and a variation of lead isotopic composition can be shown in these two environments. The lead isotopic composition of superficial lake sediments (age between zero and ten years) and of recent snows from the alpine glacier is practically identical with that produced by the local automobile exhausts and is very different from the lead isotopic composition of older ice or sediments (age ~ hundred years) which are more radiogenic (similar to natural modern leads).

A few values of lead isotopic composition in gasoline or in samples representative of automobile exhausts in France, Spain and Switzerland in the context of our study or in England by (3), seem to indicate that the particular situation met in Belgium can probably be applied to other European countries.

We propose to extend this kind of study to the lead existing in the human body, in order to evaluate the fraction of this lead resulting from the combustion of gasoline and to bring so a useful element of information for the authority which must make a decision concerning the allowable concentration of lead in the automobile gasoline.

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Along the same lines we investigated the possibility of application of this method in Belgium and neighbouring countries. For this purpose, systematic study was undertaken of the lead isotopic composition in samples of gasoline, of vehicle exhausts (air taken in roadway tunnels and vegetation and soils along highway borders) and in samples highly polluted by leads from local industrial effluents (sediments and vegetation of highly industrialized regions). Our results show that, assuming an error not larger than a few %, on the isotope ratios, it is possible in Belgium at least, to distinguish the lead emitted by automobile exhausts from the lead of other sources, either industrial or natural.

This is due to the fact that the lead alkyls additives sold on the Belgian market are manufactured, since at least several years, with leads very low in radiogenic isotopes. The lead alkyls added to Belgian gasolines are supplied by only two companies : Ethyl S.A. and Octel Co. The last and main supplier, uses only leads from Australian and Canadian ores which are of early pre-Cambrian age and thus less radiogenic.

Once this fact established, an estimation has been given of the relative contribution of lead resulting from the combustion of gasoline in different parts of the environment, principally in Belgium and, to a less extent, in neighbouring countries. Our results show that, as expected, the atmospheric lead aerosols come essentially from gasoline combustion. Moreover, this source of lead is dominant in other environments investigated : lacustrine sediments (Belgian Luxembourg), glacier ice (glacier of Tsanfleuron, Alpes Vaudoises) and suspended matter of river (Scheldt) and marine (Southern Bight of the North Sea) origin. It was found that the lead presently contained in the lacustrine and glacial environments, originate almost entirely from the atmosphere while the river and marine environments lead contains a non negligible contribution from industrial and domestic effluents. In the case of lacustrine sediments and temperate glaciers, the history of the atmospheric pollution by lead has been studied by comparing, for a same time period, the isotopic composition and the concentration of this

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Contractor : E.C. Environmental Research

Contract n° : 080-74-2-ENV.B

Project Leader : J.R. MAISIN

Title of project : Les effets morphologiques de l'action toxique du plomb
sur les organismes en voie de développement.

Our work was concerned with different aspects of lead toxicity

1. Lead effects on the microstructure of the central nervous system (cerebral cortex of the rat)
 2. Long-term toxicity and structural changes produced by lead in male germ cells in mice
 3. Lesions associated with the presence of lead in mice embryos.
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1. Lead effects on the microstructure of the central nervous system (cerebral cortex of the rat)

The nervous system is particularly sensitive to the presence of lead in the environment. Important and varied lesions were observed at this level. The purpose of this work is to study both the mechanism of development of this damage and to follow its evolution.

Various experimental approaches were devised. In this report, only short term experiments with high doses of lead (1 and 0.5 % lead ion in food as lead acetate) will be relate ; juvenile female rats (1, 2 and 3 months old) intoxicated from one week before delivery were used.

The results from rats fed with 1 % Pb^{++} are briefly reported here. All the cell types of the cerebral cortex (CC) display noticeable changes of their structure and ultrastructure.

a) Vascular lesions : appear as the most important changes in the poisoned rat. Haemorrhages, occlusion and "activation" of the capillary vessels were already noted in recent works. Moreover, we have found a large increase of the capillary density (CD) in the lead treated CC (1, 2). The convolution rate of the vessels was also increased. Similar conclusions were proposed by Gerber et al., from our department, but using the microsphere approach. In addition, we noted also the presence of a (negative) correlation between the increasing CD in the treated rats and the important decrease of their cortex thickness from 1 to 3 month-old. The ultrastructure of the vessel walls was noted to be only slightly modified : it was often characterized by enlarged cisternae of the endothelial endoplasmic reticulum.

In these conditions, a model of lead toxicity for the CC is proposed ; the nerve parenchyma would be more sensitive to the damaging action of lead and its involution would be responsible for the increase of the CD as a passive reaction. Indeed, the electron microscope confirms the presence of damaged structures at the level of nerve and glial cells.

b) Nerve cell lesions : Although generally unnoticed by previous workers in the field, careful morphometric observations are able to reveal significant changes of the fine structure of the neurons, particularly with respect to the subsurface cisternae, a typical organelle of these cells. Changes in number, form, size and "replication rate" have been noted (5). With the help of such parameters, lead treated 3 month-old animals were noted to maintain a juvenile-characterized type of nerve cell development.

On the other hand, subsurface cisternae now appear as valuable neuron bioindicators (4) and the study of their response to other pathologic agents is worth studying (X-rays, ageing, other heavy metals, opiumlike drugs).

- c) Glial alterations : The lead treated CC provides an interesting model for further research concerning the delicate oligodendroglial pathology. Oligodendrocytes (the CNS myelinating cells) are largely modified after 2-3 months lead action (3). The quantitative evaluation of the various pathological stages could provide new data about their possible relation with the microglia (CNS free phagocytes) and the controversial eventuality of their microglial transformation. The number of lysosomes and autophagosomes was seen to increase in the glial toxic cells ; this could provide an interpretation to the acid phosphatase and glucuronidase increase noted by Gerber in its biochemical approach to identically treated rats.

Longer term and late effects experiments, still under completion, were also devised to follow the evolution of the preceding changes and to evaluate the repair mechanisms in the toxic brain.

2. Long-term toxicity and structural changes produced by lead in male germ cells in mice

The principal points of this work are as follows : (6)

The effects of a dietary supplement of lead as lead acetate, ranging from 4 % to 0.1 % (% as lead) were studied in 3 month old male BALB/c mice. The following parameters were studied to assess long-term lead effects ; survival, body weight, seminal cytology. High concentrations of lead (4 % to 0.5 %) cause a dose-related adverse effect on survival, while the concentration of 0.1 % in food had no effect on survival in comparison with controls. Body weight measurements were confined to animals treated with 1.0 % and 0.1 % lead acetate. Severe weight loss was noted for 1.0 % but no effect was recorded for 0.1 %. The reductions were of the order of 50 % after 2-3 months.

While weight differences were immediately apparent after lead treatment, seminal cytology modifications were noted only after 2 months of treatment. The control value for the level of sperm abnormalities, including head and tail deformations, was of the order of 30-40 %, but lead treatment at the 1.0 % level increased this value to about 60 % after 2 months, a level which was maintained after nearly 3 months. A direct effect on sperm maturation in the testis is a possibility which might receive support from the detection of elevated levels of lead in these organs but the other possibility of an indirect effect due to under-nutrition due possibly to anorexia cannot at the present time be eliminated.

3. Lesions associated with the presence of lead in mice embryos

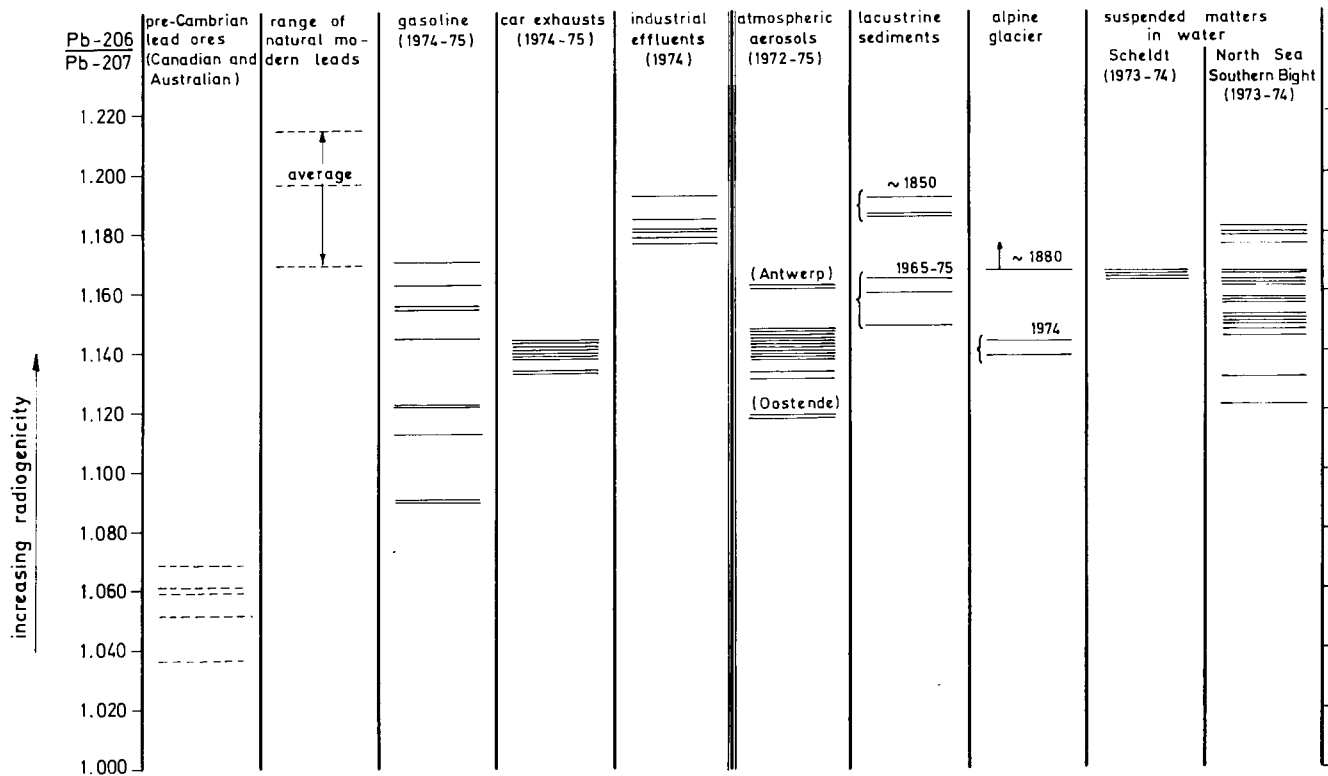
In our studies of embryonic death due to lead exposure we have confirmed in BALB/c mice the results of A. Léonard and co-workers of our department in C57Bl mice. Thus, a diet containing 0.1 % lead acetate delays the early divisions in the embryos and increases the proportion of non-dividing eggs. This lead concentration also significantly increases the percentage of embryos dying after implantation. A concentration of 0.5 % completely suppresses the fertility of female mice. Consequently a dose of 0.1 % lead in the diet was chosen to study changes in ultrastructure of the embryos. BALB/c female mice 3 months old or male mice of the same age, given a diet containing 0.1 % lead acetate during 7, 30 and 50 days before mating were caged together and then examined daily for the presence of vaginal plugs. Females with vaginal plugs were removed and killed at different times.

No qualitative changes in the ultrastructure of the cytoplasm or of the nuclei of young embryos (1 to 8 blastomeres) or of old embryos (7 to 15 days) were seen except for the presence of lead inclusions in the mitochondria and in the cytoplasm.

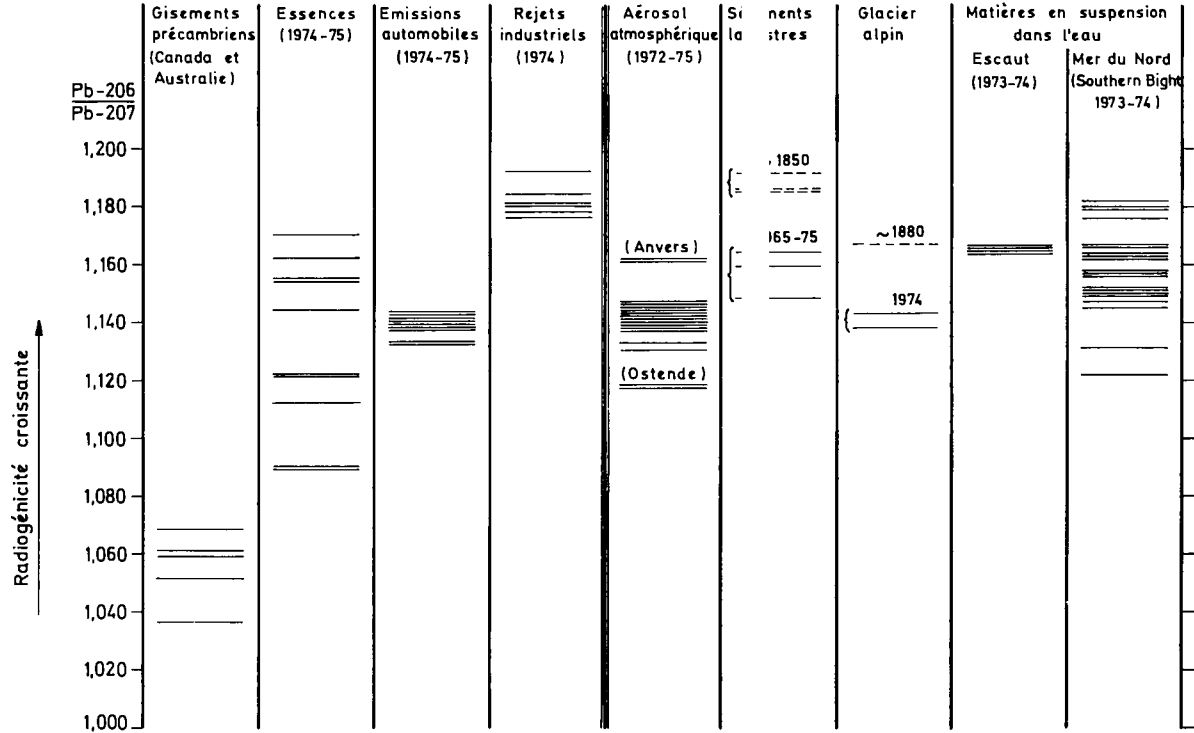
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Pb-206 / Pb-207 isotope ratio in the samples analyzed (—)
 (Belgium 1972-75) grouped by source and environment.



Rapport isotopique Pb-206 / Pb-207 dans les échantillons analysés
(Belgique 1972-75) regroupés par : source et milieu.



Contractor : United Kingdom Atomic Energy Authority (Harwell)

Contract No. O24-74-1 ENV-UK

Project Leader : Dr. A.C. Chamberlain

Title of Project: Measurement of uptake of lead from motor exhaust in the lung by means of ^{203}Pb added to petrol as tetra-ethyl lead

A method was derived for labelling tetra-ethyl lead (TEL) with the radioactive tracer ^{203}Pb . The TEL was added to petrol and burnt in an engine to produce labelled exhaust. Variations in the operation of the engine and the dilution of the exhaust were made so as to produce a range of particle sizes in the lead aerosol.

Volunteer subjects inhaled the radioactive exhaust. The deposition in the lung, transfer to blood and excretion of the ^{203}Pb was measured. Also, comparative experiments were done in which ^{203}Pb was given intravenously, either in saline solution or attached to red blood cells. Some measurements of uptake from the gut were also made.

The main findings were as follows:

The percentage deposition depended on the particle size and on the respiratory parameters, particularly the length of the respiratory cycle. For a cycle of 15 breaths/min, the percentage deposition varied from 14% for coagulated aerosols with mass median diameter $0.75\ \mu\text{m}$ to about 60% for diluted primary aerosols with particles in the 0.01 to $0.03\ \mu\text{m}$ range. For all aerosols studied, deposition was mainly in the pulmonary region.

The ^{203}Pb activity in the lung was cleared with an initial half life of about 7 hours. Not more than a few percent was retained in the lung beyond 48 hours. The appearance of ^{203}Pb in the blood followed a similar time sequence, with maximum about 30 hours after inhalation. Thereafter, ^{203}Pb in blood declined with a half life of 16 days. Of the ^{203}Pb initially deposited in the lung, about 50% was present in blood at 30 hours. The remainder was transferred to other tissues in the body. A similar percentage uptake in blood was found after intravenous injection. From this and other work it is known that a rapid partition of tracer doses of

lead between red cells, bone and other storage sites, and extra-cellular fluids, occurs in the first hour after entry of lead into blood plasma.

Excretion of both inhaled and injected ^{203}Pb was found to be distributed between urine and feces in the ratio 1:0.7. The finding of substantial endogenous fecal excretion is important in other connections, for example in estimating uptake of lead from the gut by comparison of urinary output with oral intake.

From the work it was concluded that the elevation in blood lead to be anticipated in an adult exposed to $1 \mu\text{g}/\text{m}^3$ (24 hour average) of lead in air, as a result of uptake in the lung, was about $1 \mu\text{g}/100 \text{ ml}$, with a possible variation in the range from 0.5 to $2 \mu\text{g}/100 \text{ ml}$ depending on the particle size of the aerosol and other variables.

Publications

Chamberlain, A.C., Clough, W.S., Heard, M.J., Newton, D., Stott, A.N.B. and Wells, A.C. Uptake of lead by inhalation of motor exhaust. Proc. Roy.Soc.Lond.B., 192, 77-110 (1975)

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Contractor : University of Glasgow, Department of Materia Medica

Contract No. 032 - 74 - 1 ENV UK.

Project Leader : Professor A. Goldberg/Dr. M.R. Moore



Title of project : Studies on the biological effects of lead and cadmium in the environment.

1. Role of lead and cadmium in ischaemic heart disease

There is no known cause for the increased mortality due to ischaemic heart disease in soft water areas. Since the lead concentration of soft water is often elevated, studies have been carried out to ascertain whether excess lead in drinking water is a causative factor. Rats were given water ad libitum containing lead at concentrations similar to those found previously in Glasgow, which has a soft water supply. There was increasing deposition of lead in the heart and other organs of these animals, associated with a fall in cardiac activity of the enzymes ferrochelatase and delta-aminolaevulinic acid dehydratase, which changes become maximal after six months in the highest dose group (1000 µg/l). Rats in the lower dose groups showed no ultrastructural abnormality of cardiac papillary muscle at any time during the experiment. In the highest dose group (1000 µg/l) there was marked ultrastructural change seen at 25 weeks. At this time alterations have been observed in the villous structure of the rat jejunum as shown by scanning electronmicroscopy. The studies suggested that there might be some accommodation to dietary lead after 25 weeks in this highest dose group (1000 µg/l). This could not be confirmed in studies measuring the retention of a tracer dose of lead (^{203}Pb).

A comparison of lead uptake as ^{203}Pb between the control and 1000µg/l groups over 3 days showed no significant difference in the ratio of uptake of lead into the various organs or into the cardiac organelles. A similar experiment carried out using cadmium showed no significant change in the enzymes of haem biosynthesis within the heart.

2. Lead, cadmium and hypertension

Lead It has long been felt that exposure to lead may be related to the development of systemic hypertension. A study was carried out on the island of Tiree in the Inner Hebrides of Scotland, where there is

a high incidence of hypertension. It was found that 90 per cent of the 110 water samples examined were below the WHO limit of $100\mu\text{g}/\text{l}$, and that the remainder had only minimal elevations of water lead content. It was concluded that there was no association between hypertension and water lead content in Tiree.

In the Burghs of Paisley and Renfrew, domestic water is drawn from two different sources. We have shown marked differences in the plumbosolvency of these supplies. This provided an opportunity for an internally-controlled experiment to find the influence of lead from domestic water on blood pressure and other aspects of renal and cardiac function. We identified 150 individuals with hypertension, detected in the course of the Midspan Health Survey. Samples of blood and domestic water were obtained from these subjects and from an age and sex matched Control group, and analysed for lead content.

The results from 135 matched pairs of male and female hypertensives and normotensives indicated a significant association between blood lead concentrations and hypertension in males ($p < 0.05$). Females showed a similar distribution which did not achieve statistical significance.

Cadmium has also been thought to be a factor in the aetiology of hypertension. It has been shown previously that there was significantly higher urinary cadmium excretion in hypertensives. In 70 hypertensive patients and 70 matched controls investigated for a possible relationship between blood cadmium and hypertension, no significant difference was shown between the two groups. The blood cadmium concentrations were significantly higher in smokers as compared with non-smokers. These results do not support the hypothesis that cadmium is involved in the development of hypertension in man.

3. The role of water lead exposure in mental retardation

In a study of the role of chronic low level lead exposure in the aetiology of mental retardation, we have measured water lead levels in homes occupied during the first year of life by 77 mentally retarded children aged 2-6 years, and 77 non-retarded children matched for sex and age and social class. Also examined were the homes occupied by these children's mothers during pregnancy. It was found that the distribution of water lead concentrations indicated a significantly higher 'tail' of high water lead concentrations in the children with mental retardation and that the probability of mental retardation was significantly increased when water lead exceeded $800\mu\text{g}/\text{litre}$. If all that was known about exposure to water lead for a certain child was that it exceeded $800\mu\text{g}/\text{l}$, the statistical conclusion of these studies

is that the child was at least 1.7 times more likely to be defective than a child whose exposure to water lead was completely unknown. Where blood lead levels were able to be measured it was found that these were significantly higher in the retarded group and from these studies it was concluded that lead contamination of water is one factor in the multifactorial aetiology of mental retardation.

Work carried out on cord blood concentrations, maternal blood lead concentrations and domestic water lead concentrations have demonstrated highly significant regressions between these parameters. Studies have shown that the concentrations of lead in breast milk, which are the same as those in blood plasma, correlate significantly with whole blood lead concentrations of maternal and cord blood.

4. Studies on lead absorption: influence of calcium

There is continuing controversy over the role of calcium as a prophylactic agent in lead exposure. In the present studies we have doubled the dietary calcium of rats using the calcium salts, gluconate, glycerophosphate, orthophosphate, carbonate, chloride and sulphate. These caused highly significant depressions in lead absorption as assessed by whole body retention of an oral dose of 50 μCi ^{203}Pb chloride. There was no significant difference between calcium and control groups in retention of an intraperitoneal dose of ^{203}Pb . Milk had no influence on the rate of lead absorption from the gut. Further studies revealed that rats administered lead in water hardened artificially with calcium chloride to levels found in nature, absorbed less lead than animals administered lead in distilled water. Calcium can limit oral lead absorption, this being important in soft water areas where lead uptake may be increased not only by increased plumbosolvency but also by increased absorption.

An industrial trial has now been completed in a group of workers in a secondary lead smelter in Glasgow. In this crossover trial the workers took 1g calcium glycerophosphate or placebo twice a day, in an attempt to limit the uptake of lead. Blood lead levels, erythrocyte delta-aminolaevulinic acid dehydratase, urinary delta-aminolaevulinic acid and coproporphyrin were measured. It was found that calcium glycerophosphate significantly depressed the blood lead concentrations and raised blood delta-aminolaevulinic and dehydratase activity and lowered blood ALA concentrations of the workers utilising

this preparation. These results suggest a possible use of calcium glycerophosphate in the prophylaxis of industrial lead exposure.

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Contractor: Department of Occupational Health,
University of Manchester

Contract No: O33-74 - 1 ENV - UK

Project Leader: Professor W.R. Lee

Title of Project: A clinical and biochemical study of early lead poisoning

Aim

The purpose of this study was to look for any relationship between alteration in nerve conduction in lead workers, and some of the commonly used tests of lead absorption and consequent disturbance of haemopoiesis.

Introduction

Disturbance of peripheral neuromuscular function in lead poisoning (eg 'wrist drop') has been recognised for several centuries and, more recently, a number of studies have shown a decrease in motor nerve conduction velocity in persons working with lead. However, no studies have demonstrated a close relationship between these changes in the blood lead level or some other indices of disturbed haemopoiesis such as urinary ALA and free erythrocyte porphyrins. The present investigation was, therefore, undertaken to see if any other of the commonly used indices of lead absorption, generally from disturbed haemopoiesis, were related to alteration in motor nerve conduction velocity.

Preliminary Studies

These were carried out to determine the possible influence of factors which might complicate the results. These factors included variability, both "within subject" and "between subject", age, dominance (handed-ness), skin temperature at times of testing, and sex.

The "within subject" variability on repeated testing was found to be about 5% and the "between subject" variability about 10%. (In the event rigorous attention to experimental detail kept the coefficient of variation of motor conduction velocity of both lead workers and controls to less than 10%, apart from the radial nerve.) Tests on peroneal motor conduction velocity on twenty subjects, aged from 19 - 67 years demonstrated, as expected, a clear inverse correlation with age. It was decided, therefore, to match each exposed lead worker with an age matched non exposed control. Dominance appeared to have no significant effect on any of the parameters measured. Variations in conduction velocity due to changes in skin temperatures were found to be negligible when these temperatures were within the ranges used during the study ($>33^{\circ}\text{C}$ proximally, $>31^{\circ}\text{C}$ distally in the arm, and $>31^{\circ}\text{C}$ proximally, $>30^{\circ}\text{C}$ distally in the leg). No maximum temperature was set, as increasing temperature does not appear to affect conduction velocity significantly. There appeared to be no significant difference between age-matched males and females. However, in view of reports in the literature of differences in the conduction velocities of men and women, it was decided to confine the study to men.

Method

Ninety-four lead workers and ninety-four age matched controls were examined. All subjects were screened by questionnaire to eliminate those with disease or injury of the nerves or with anaemia.

The following indices of peripheral nerve function were measured:

Maximum motor conduction velocity (MMCV) was measured in the ulnar, median, radial and peroneal nerves.

Sensory conduction velocity (SCV) in the ulnar nerve.

Percentage Amplitude (amplitude of muscle action potential, MAP, after proximal stimulation expressed as percentage of MAP amplitude after distal stimulation) was taken as an indication of slow fibre conduction velocity (SFCV). It was measured in the ulnar median and peroneal nerves.

The following biochemical indices were measured:

Blood lead (Pb-B); Erythrocyte ALA-D; Free erythrocyte porphyrins (FEP); Haemoglobin (Hb); Urine lead (Pb-U) (corrected to standardised osmolality of 1024); Urinary ALA.

Findings (Table 1)

MMCV The study confirmed that all MMCV were highly statistically significantly lower in the lead exposed group.

SCV As anticipated, there was no statistical difference between the two groups.

Percentage Amplitude was reduced in lead workers in the peroneal but not in the median or ulnar nerves. It may not be an appropriate indication of SFCV in these latter two nerves because of cross stimulation.

There was no statistically significant correlation between nerve conduction velocity and present exposure to lead as measured in a three point scale determined by present lead work or as measured by any of the biochemical indices. (See Table 2).

Multiple regression analysis, to show the contribution of length of exposure (whilst taking into account of the effect of age),

showed a highly significant statistical correlation between increasing length of exposure and decreasing nerve conduction velocity.

An important observation was that in thirteen men who had been exposed to lead for two years or less and whose blood lead levels had not risen above $80 \mu\text{g}/100 \text{ ml}$ in three monthly tests (mean Pb-B at time of testing, $58 \mu\text{g}/100\text{ml}$), the MNCV of the ulnar, radial and peroneal nerves and the peroneal percentage amplitude were significantly reduced.

Conclusions

This study confirmed that in a large group of asymptomatic lead workers, the maximum motor conduction velocity and possibly also the slow fibre conduction velocity is reduced when compared with matched non-exposed controls. Length of exposure could be demonstrated, statistically, as a factor in this, but severity of present exposure apparently could not. This latter is in accord with the finding that not one of the six indices of disturbed haemopoiesis was correlated with change in nerve conduction velocity.

Table 1: PAIRED 1-TAILED T-TEST: LEAD WORKERS COMPARED WITH CONTROL SUBJECTS

Measurement	N	Mean of Lead Workers ± S.D.	Mean of Controls ± S.D.	P	SIG
Age	94	39 ± 9	39 ± 9	-	N.S.
Blood Lead	85	60 ± 15	24 ± 9	<0.0005	SIG
Ulnar Motor Conduction Velocity	94	53.4 ± 4.1	55.6 ± 4.3	<0.0005	SIG
Median Motor Conduction Velocity	94	55.9 ± 3.9	57.3 ± 3.9	<0.01	SIG
Radial Motor Conduction Velocity	91	63.9 ± 12.9	71.7 ± 10.1	<0.0005	SIG
Peroneal Motor Conduction Velocity	91	46.1 ± 3.7	47.6 ± 4.0	<0.005	SIG
Ulnar Sensory Conduction Velocity	76	57.5m.s. ⁻¹ ± 4.1	57.9m.s. ⁻¹ ± 4.9	>0.05	N.S.
Ulnar Nerve Action Potential	92	61.7m.s. ⁻¹ ± 4.7	61.8m.s. ⁻¹ ± 5.3	>0.05	N.S.
Ulnar % Amplitude	94	92.2 ± 7.4	92.6 ± 9.7	>0.05	N.S.
Median % Amplitude	94	94.2 ± 7.3	94.2 ± 9.3	>0.05	N.S.
Peroneal % Amplitude	91	86.6 ± 10.8	90.3 ± 7.9	<0.005	SIG

Table 2: Pearson Correlation: nerve measurements and biochemical measurements (lead workers only)

Correlation between	N	r	Slope	P	Significance (at 95% level)
MEDIAN MAXIMUM MOTOR CONDUCTION VELOCITY <u>AND</u>					
Blood lead	91	0.018	0.005	0.864	Not significant
Aminolaevulinic acid dehydrase	94	0.148	-0.142	0.156	"
Protoporphyrin	91	0.092	0.006	0.388	"
Haemoglobin	94	-0.115	-0.0536	0.269	"
Urinary aminolaevulinic acid	72	-0.028	-0.017	0.813	"
Urine lead	92	0.182	0.152	0.083	"
Corrected urine lead	86	-0.075	0.044	0.495	"
RADIAL MAXIMUM MOTOR CONDUCTION VELOCITY <u>AND</u>					
Blood lead	88	-0.089	-0.080	0.412	"
Aminolaevulinic acid dehydrase	91	0.154	0.480	0.145	"
Protoporphyrin	88	-0.110	-0.245	0.306	"
Haemoglobin	91	-0.051	-0.785	0.630	"
Urinary aminolaevulinic acid	69	0.046	0.088	0.710	"
Urine lead	89	-0.154	-0.417	0.150	"
Corrected urine lead	83	-0.072	-0.135	0.520	"
PERONEAL MAXIMUM MOTOR CONDUCTION VELOCITY <u>AND</u>					
Blood Lead	89	0.153	0.040	0.153	"
Aminolaevulinic acid dehydrase	92	-0.071	-0.065	0.502	"
Protoporphyrin	89	0.056	0.004	0.601	"
Haemoglobin	92	-0.051	-0.229	0.632	"
Urinary aminolaevulinic acid	70	0.004	0.002	0.973	"
Urine lead	90	0.122	0.099	0.251	"
Corrected urine lead	84	-0.016	-0.001	0.885	"
PERONEAL PERCENTAGE ANPLITUDE <u>AND</u>					
Blood lead	89	0.043	0.032	0.689	"
Aminolaevulinic acid dehydrase	92	0.080	0.213	0.448	"
Protoporphyrin	89	0.158	0.030	0.141	"
Haemoglobin	92	-0.079	-1.029	0.457	"
Urinary aminolaevulinic acid	70	0.169	0.236	0.161	"
Urine lead	90	0.103	0.238	0.336	"
Corrected urine lead	84	0.167	0.267	0.129	"

Contractor: City of Birmingham Environmental Services Committee
and University of Aston in Birmingham.

Contract No. 105 - 75 - 1 - ENV - UK.

Project leader: Professor John A. Blair.

Title of Project: Intestinal Absorption of Lead Compounds.

Food and water are the major sources of lead compounds in man. However, the mechanisms by which lead compounds are transferred from the intestinal lumen across the gut wall are not understood; nor is it known whether lead compounds interfere with the transport of other nutrients or with intestinal processes. We now report a study of the transport of lead compounds across the gastrointestinal tract and their effects on a number of different physiological processes.

1. TRANSPORT OF LEAD AND ITS EFFECT ON INTESTINAL FUNCTION

The Wilson and Wiseman everted sac technique was used to investigate the transport of lead compounds across the intestine of male Wistar rats (weight approx. 250g.). To assess preparation viability the P.D. across seventeen successive sacs from the small and large intestine was measured. The potential difference measured at 10 minute intervals across preparations incubated in Krebs-Hensleit buffer containing 20mM glucose at 37°C was stable over one hour and varied from 4.4mV (distal duodenum) to 9.0mV (distal jejunum) and 5.2mV (mid colon) thus establishing preparation viability. Addition of 10^{-5} or 10^{-6} M lead acetate (concentrations approximating to that in the adult intestinal lumen from dietary lead) to both mucosal and serosal solutions resulted in small but not statistically significant changes in the P.D. measured similarly to the control sacs.

Using the same physiological preparation and the same concentrations, the mucosal effect of lead acetate on glucose and water transport was investigated. The serosal-mucosal glucose concentration ratios in lead free solutions after one hour incubation varied from 1.2 (proximal duodenum) to 1.8 (distal jejunum). The ratio was lowered across all preparations after addition of 10^{-6} M lead acetate to both serosal and mucosal solutions. However, the reduction was

only significant across the lower jejunum in the presence of 10^{-5} lead acetate.

Water transport measured at ten minute intervals over one hour proceeded in a linear fashion across all the everted sac preparations. Total water transport varied from 480mg/g tissue/hour (duodenum) to 1500mg/g tissue/hour (jejunum) to 660 mg/g tissue/hour (colon). In the presence of 10^{-6} M and 10^{-5} M lead acetate mucosally, water transport was virtually unaffected.

Transport of lead acetate from the mucosal solution was estimated using the radioisotopes ^{210}Pb and ^{203}Pb as tracers and at 10^{-7} M, 10^{-6} M and 10^{-5} M lead acetate concentration. Lead appeared in the serosal solution at much the same rate in all parts of the intestine. Measured at ten minute intervals over one hour it was linear with time at 10^{-7} M and 10^{-6} M; at 10^{-5} M a rate decrease appeared after 20 minutes. At 10^{-5} M the rate of transport across the mid jejunum into the serosal space was about 58 ng Pb/g wet weight tissue/hour. The final concentration of lead in the serosal space in all sacs never exceeded 4% of the initial mucosal concentration. A plot of the serosal uptake of lead measured at ten, twenty, and sixty minutes at 10^{-7} M, 10^{-6} M and 10^{-5} M lead acetate showed a linear increase with concentration, without any evidence for a saturatable process. At mucosal concentrations of 10^{-6} M and 10^{-5} M lead acetate uptake occurred rapidly on to the tissue in sacs from all sites of the intestine being complete within 10-20 minutes and thereafter remaining constant up to sixty minutes. At 10^{-5} M concentration maximum mucosal lead uptake for the mid jejunum was 12.1 $\mu\text{g Pb/g}$ wet weight tissue. At 10^{-7} M lead mucosal concentration tissue uptake of lead was linear with time and showed no evidence for saturation. The tissue uptake of lead at 10^{-7} M, 10^{-6} M and 10^{-5} M lead acetate was linear with concentration and showed no evidence for a saturatable process.

It is concluded that at low levels of lead similar to that likely to be ingested, lead is transported slowly out of gut lumen by passive transport mechanisms, lead is rapidly and strongly bound to the intestinal mucosa and causes no marked effects on water, sodium or glucose transport across the intestine. The decrease in the rate of serosal uptake of lead at 10^{-5} M concentration and a reduced S/M ratio for glucose at 10^{-5} M in the lower jejunum suggests a possible effect at this elevated concentration. The continuing tissue uptake of lead at 10^{-7} M concentration contrasted with the rapid saturation of lead uptake at the higher concentrations of 10^{-6} M and 10^{-5} M may suggest a possible toxic effect at 10^{-6} M lead concentrations which may still persist in some

areas of the gut at $10^{-7}M$ lead.

Coeliac disease and Crohn's disease are intestinal disorders associated with malabsorption of nutrients. Measurement of the blood lead levels of subjects with these conditions and comparison with the normal Birmingham population showed no significant differences (males, normals $21.8\mu\text{g}/100\text{ml}$ (221), coeliacs $19.6\mu\text{g}/100\text{ml}$ (12), Crohn's $23.7\mu\text{g}/100\text{ml}$ (12); females, normals $14.8\mu\text{g}/100\text{ml}$ (216), coeliacs $16.7\mu\text{g}/100\text{ml}$ (37), Crohn's $16.2\mu\text{g}/100\text{ml}$ (38). Some coeliacs have a special gluten free diet; their blood lead levels were not significantly different.

As large amounts of lead compound are taken up by the mucosal intestinal surface it seemed likely that this might affect surface properties and enzyme activities. However, lead acetate at up to $10^{-3}M$ mucosal concentration had no effect on (i) the acid microclimate at the mucosal surface of the intestine, or (ii) the activity of the brush border enzymes lactase and trehalase.

2. MODELS RELATING BLOOD CONTENT OF LEAD TO DIETARY INTAKE OF LEAD AND RED BLOOD CELL COUNT

These intestinal absorption studies show lead to be slowly absorbed from the gut lumen at all sites in the intestine by a passive process. Lead compound concentration in the lumen of the intestine for the average person in the U.K. is estimated to be approximately $40\mu\text{g}/100\text{mls}$ (Daily intake of lead compounds in food and beverages approx. $200\mu\text{g}/\text{day}$; volume of gut fluid approx. 500mls). Of this probably 90-95% is bound to the mucosal surface as judged from rat mucosal surface absorption. The free lead concentration is about $2\mu\text{g}/100\text{ml}$ fluid. The Birmingham mean blood lead level of adult males is $21\mu\text{g}/100\text{mls}$ whole blood. As about 95% of the lead is attached to the red blood cells, which occupy 45% of the total blood volume, the concentration of unbound lead is therefore about $2\mu\text{g}/100\text{ml}$ serum. The agreement between free serum lead concentration and free lead concentration in the gut fluid are what would be expected for a passive transport process. They suggest that the gut free lead concentration is the controlling feature of blood and soft tissue lead levels.

Measurement of the blood lead concentration and red blood cell count in a group of healthy hospital staff (40) aged under 25 years gave a good linear correlation. Thus in considering blood lead levels the haematocrit level should also be determined. Two groups of subjects probably have low blood

lead levels due to reduced haematocrit; menstruating women (women aged 25-34 yrs 14.4 μ g/100 ml (62); men aged 25-34 yrs 22.4 μ g/100 ml (89)) and anaemic subjects (blood lead: males 10.0 μ g/100 ml (11); females 8.3 μ g/100 ml (15)). The correlation between blood lead and haematocrit levels suggests that above blood lead levels of 30 - 35 μ g/100 ml, the free lead concentration must rise rapidly giving rise to toxic effects.

3. BIOCHEMICAL EFFECTS OF LEAD COMPOUNDS

Recent reports have suggested that there may be an association between raised body burdens of lead and hyperactivity or mental retardation. In the latter case the ingestion of high concentrations of lead from drinking water has been implicated. In an attempt to relate these observations to a biochemical phenomenon, the effect of lead on tetrahydrobiopterin metabolism was investigated. A defect in biopterin metabolism would indirectly interfere with the synthesis of the neuro transmitters dopamine, noradrenaline, adrenaline and serotonin. Rats were injected IP with lead acetate, and serum biopterin levels determined using a *Crithidia fasciculata* assay. Serum biopterin levels were significantly reduced following each injection of an acute dosage of lead (300 g Pb/Kg body weight). However, further investigations using lower concentrations of lead are needed before accurate deductions can be made concerning the relationship between the subclinical effects of lead and biopterin levels.

Contractor : University of Odense (previous residence of project leader)

Contract n° : 025-74-1 ENV DK

Project leader : Prof. Jørgen Clausen, Department of Biochemistry and Toxicology, Institute of Life Science, Roskilde University, Chief of Neurochemical Institute, Copenhagen

Title of project: Heavy metal levels and δ -amino levulinic acid dehydratase (ALA-D) activity in normal Danes and in peripheral poly-neuropathy.

The heavy metal levels (lead, cadmium, nickel, manganese, copper and chromium) were assayed in 57 normal individuals. The normal values were presented as mean and median values and compared to the activity of δ -amino levulinic acid dehydratase activity (ALA-D). Only for lead a significant inverse correlation could be demonstrated to ALA-D activity. The heavy metal levels were higher among men than among women.

The validity of the correlation between ALA-D activity and lead level in whole blood was calculated. At the limit value for blood lead: 80 ug/100 ml, high specificity but low sensitivity was revealed. However, ALA-D activity in blood was found to be more relevant marker for lead levels at the limit 40 or 60 ug/100 ml compared to 80 ug/100 ml (Table 1).

On the basis of the above mentioned normal data the heavy metal levels were studied in 23 patients with peripheral neuropathy on unknown etiology.

All patients studied showed electro-physiological signs of denervation and/or reduced motor or sensoric nerve conduction velocity. Cadmium and manganese were never found to be increased. In all but four patients, an increase of one or more heavy metals was found. Ten patients showed raised levels of two or more metals, the dominant metal being lead (10 cases), nine patients showed increased nickel values, eight increased copper values and two an increase in chromium. Although normal concentrations of manganese were found in this group of patients, correlation analysis revealed a significant correlation between increased manganese and decreased ALA-D. The raised values of heavy metals could not be traced to occupational factors or other exposure to heavy metals in the homes and the increased values were not related to tobacco consumption. The findings are discussed in relation to known data on peripheral neuropathy and the results seem to indicate a multifactorial pathogenesis of this group of diseases.

Among factors contribution to the precipitation of the syndrome may be raised levels of heavy metals.

Furthermore the heavy metal pollution was assayed in 216 individuals working in ten garages on the island of Funen and related to data from biochemical and medical examinations. Clinical symptoms were recorded by means of questionnaire. Increased blood lead levels were found in 59 % with 9 % having above 80 ug lead/100 ml (3.86 umol) whole blood. Mechanics in eight out of ten garages had significantly increased blood lead levels. A decrease in δ -amino levulinic acid dehydratase (ALA-D) activity was associated with increased blood lead levels but the latter were not related to haematological changes, tobacco consumption or to length of service in the trade. Particulate lead air pollution was not the sole cause of increased blood lead levels. Raised lead values were maximal among diesel engine workers who are exposed to high pressure-resistant lubricants containing lead naphthenate. As these workers complained of skin damage, lead absorption may have occurred through the skin. Assay of lead content showed 9290 ppm in gear oil and 1500-3500 ppm in used motor oils. The data are discussed in relation to the occupational risks in auto repair shops.

Blood of autoworkers with high lead content was also analysed for cadmium, chromium, copper, manganese, nickel, ALA-D activity and carboxyhaemoglobin level. Cadmium and copper levels in blood of autoworkers were comparable with those of the control subjects while chromium and nickel levels were significantly higher ($p < 0.01$ for both metals), and scattered raised values of manganese were found. There was no significant mutual correlation between levels of various heavy activity ($p < 0.1$). Nineteen per cent of autoworkers were found to have an abnormally high blood level of carboxyhaemoglobin. The amount of particulate heavy metal in autoworkshop air was not related to biochemical abnormalities found in the autoworkers. Various sources of pollution of these heavy metals in autoworkshops are discussed.

Twenty of the automechanics possessing increased whole blood values of one or more of the following heavy metals: chromium, copper, lead, manganese and nickel, were studied for peripheral nerve affection by means of electromyography (both sensoric and motoric nerve potentials were recorded). The heavy metal contents were related to the findings of denervation, distal motor latency, distal sensory latency, motoric and sensoric conduction velocities. Apart from two workers, in whom only lead was assayed, the remaining

group of 18 were assayed for all heavy metals under study. Six workers showed increased distal motor and/or sensory latency and seven decreased nerve conduction velocity (four motoric and three sensoric affections). Of the workers with nerve affection, three showed increased levels of lead (nickel and chromium also raised). Four workers showed increased lead, nickel and chromium and one of lead, chromium and manganese. All in all, 10 out of 20 workers (50 per cent) with elevated lead levels showed definite signs of peripheral neuropathy and seven out of 14 with raised nickel values showed these signs but they could all be accounted for by the increased lead level. All except seven workers with raised lead levels in the whole group showed values above the critical limit of 80.0 ug/100 ml in whole blood. The data argue for the highly toxic effect of lead and other heavy metals on the peripheral nervous system and stress the diverse toxic exposure wich automechanics undergo during their work. The possibility of there being a synergistic action between heavy metals and components of mineral oil and petroleum is discussed.

Table 1.

The relationship between increased Pb level and depressed ALA-D in whole blood.

	lead level:		
	40 ug% (1.93umol/l)	60 ug% (2.9 umol/l)	80ug% (3.86 umol/l)
Sensitivity:	1.00	0.75	0.32
Specificity:	0.63	0.91	0.96
Validity:	1.63	1.66	1.29

Litterature Published:

1. Melgaard, B., Clausen, J. & S.C. Rastogi: Heavy metal levels and delta-amino-levulinic acid dehydrase levels in peripheral polyneuropathy. *Acta neurol. Scand.* 53:291-307, 1976.
2. Idem: Electromyographic changes in automechanics with increased heavy metal levels. *Acta neurol. Scand.* 54:227-40, 1976
3. Clausen, J. & S.C. Rastogi: Heavy metal pollution among autoworkers. I. Lead. *Brit. J. Industr. Med.* 34:208-215, 1977
4. Idem: Heavy metal pollution among autoworkers. II. Cadmium, chromium, copper, manganese and nickel. *Brit. J. Industr. Med.* 34:216-220, 1977.

TOPIC 3 : HEALTH EFFECTS OF MICROPOLLUTANTS

Contractor: Krankenhaus Bethanien für die Grafschaft
Moers, D-413 Moers

Contract n^o: 014-74-1 ENV D

Project leader: Priv.-Doz.Dr.G.von Nieding
Dr. H. M. Wagner

Title of Project: Studies on the combined effects of NO₂,
SO₂ and O₃ on human lung function.

1.1 Subjects, Concentrations Used and Exposure Unit

A group of 11 healthy male volunteers (aged 20 to 38 years) were exposed to 0 (= control), 5 ppm NO₂* (9 mg/m³), 5 ppm SO₂* (13 mg/m³) and 0.1 ppm O₃* (0.2 mg/m³) alone, to a combination of 5 ppm NO₂ + 5 ppm SO₂, 5 ppm NO₂ + 0.1 ppm O₃ and to a combination of NO₂, O₃ and SO₂ in MAK* and MIK** concentrations in an exposure chamber (MACROLON^R, about 8 m³) with constant temperature (22°C) and humidity (55 rel %) (WEISS, Giessen, type RB 700). Complete change of air in the chamber was performed about every 3 min. To eliminate the influence of circadian rhythm on lung function all experiments were carried out at a defined time of the day. The total exposure cycle consisted of a prephase (1 h), an exposure phase with intermittent light physical exercise (2 h) to double the breathing minute volume (4 times for 15 min), and a post-exposure period (1 h).

* (according to DFG-report n^o XI, 1975)

** (according to short-term values (24 h), VDI-Richtlinie 2310, 1974)

1.2 Lung Function Analysis

The pre-exposure phase was necessary to adapt the subjects to the environmental conditions within the exposure unit and was used to attain the steady state necessary for the measurement of the initial lung function values, i.e. respiratory gas exchange for oxygen and carbon dioxide (PaO₂, PAO₂, PaCO₂, PACO₂ and pH_a), airway resistance (R_{aw} measured as R_t) and thoracic gas volume (TGV). The instruments used were: a respiratory mass spectrometer (VARIAN MAT), platinum or glass electrodes (ESCHWEILER) and a volume constant body plethysmograph (own construction). Measurement of lung function parameters was made at the end of the prephase, after 1 and 2 h of exposure to air pollutants, and 1 h after

termination of exposure. In addition, control experiments under identical humidity and temperature conditions were performed.

1.3 Analysis of Pollutants and Background Concentrations of NO₂, SO₂, O₃ and Total Suspended Particles

The method considered highly specific for ozone and to be technical reliable for continuous analysis was the chemiluminescent procedure (BENDIX Ozone Monitor); calibration was done with the neutral buffered potassium iodide method (VDI-Richtlinie 2468, 1974). For the continuous analysis of NO₂ the colorimetric analyzer 'PICOS' (HARTMANN & BRAUN) was used, calibrated and checked intermittently by the colorimetric SALTZMAN (Anal.Chem. 26, 1949 (1954)) procedure. The SO₂ concentrations in the chamber were monitored by a 'Total²Sulfur Analyzer' (BENDIX). As the analytical procedure is not entirely specific for SO₂ a 'H₂S-scrubber' (BENDIX) was added to the analytical² setup. Additionally, the SO₂ levels were checked by the manual method of WEST and GAEKE as standardized by the VDI (VDI-Richtlinie 2451, 1968). The concentrations in the chamber varied between ± 10% for NO₂ and SO₂, and ± 20% for O₃, during the exposure.

Background concentrations of the pollutants NO₂, SO₂ and O₃ were determined several times under experimental conditions with a mean value of 10 ppb for NO₂ and 20 ppb for SO₂; for O₃ there was no measurable background.

Total suspended particles were measured by a high volume sampler type GRAVICON (SARTORIUS). Particle size distribution as measured with the ANDERSON impactor showed the main peak of the particles to ly in a size range of 2 μ and a second very low at very small particle sizes of < 1 μ. Total background level was 0.15 mg/m³.

1.4 Exposure to Acetylcholine

Reaction to acetylcholine challenge was studied in a control series and in two series with combined pollutants in MAK- and MIK-concentration (n = 9). The challenge was done at the end of the 2 h exposure period with 1, 2 and 3% acetylcholine solutions. The subjects started with breathing the 1% solution for 1 min by an aerosol generator (HEYER) with a standardized breathing frequency of 20/min with the tidal volume kept nearly constant. Resistance measurements were done from the 2nd to 5th minute after begin of the ACH-inhalation. Immediately thereafter the challenge with the 2% and then 3% ACH-solution was performed in the same mode.

1.5 Statistical Evaluation

The statistical evaluation was made with WILCOXON's ranking method of pair differences (Biometrics 1, 80 (1945)), where the zero hypothesis (= no effect) was tested one-sided. To

increase the power of the test PaO_2 changes below 5 mmHg and resistance increases below $0.5 \text{ cmH}_2\text{O}/(1/\text{s})$ were regarded as zero.

After calculation of significances this way, significances were recalculated implying additionally, that each subject had to leave by a certain amount the individual S.E. range of lung function parameters of interest, which was calculated for each subject from up to 9 subsequent experiments on different days, i.e. 5 mmHg for the PaO_2 -decrease and $0.5 \text{ cmH}_2\text{O}/(1/\text{s})$ for the R_t -increase, respectively. Both p-values are given in the table 1.

2. Results

Depending on criteria for statistical evaluation, i.e.

a. PaO_2 -decrease $> 5 \text{ mmHg}$ and R_{aw} -increase $> 0.5 \text{ cmH}_2\text{O}/(1/\text{s})$
or

b. PaO_2 -decrease $> 5 \text{ mmHg}$ and R_{aw} -increase $> 0.5 \text{ cmH}_2\text{O}/(1/\text{s})$
and additionally moving from the individual S.E. range
for the same amount

the following results were obtained:

1. Applying the stronger criteria, there is a significant PaO_2 -decrease and R_{aw} -increase in all series with NO_2 in MAK-concentration as compared to the initial values and the behaviour in the controls.
2. The combination of $\text{NO}_2 + \text{O}_3$, $\text{NO}_2 + \text{SO}_2$ or $\text{NO}_2 + \text{O}_3 + \text{SO}_2$ did not show a stronger effect than NO_2 alone; however, in the latter series recovery of PaO_2 seems to be delayed and R_{aw} even increases in the post-exposure period.
3. Exposure to $\text{NO}_2 + \text{O}_3 + \text{SO}_2$ in MIK-concentration did not show an effect on PaO_2 and R_{aw} .
4. Applying the less stronger criteria there are significant changes of PaO_2 in all series as compared to the initial values and the behaviour in the controls and of R_{aw} except in the SO_2 -series.
5. Acetylcholine challenge with 1, 2 and 3% solutions following the exposure to a combination in MIK-concentration resulted in a significant increased reability to the 2% ACh-solution as compared to the control series.

		Control	NO ₂	O ₃	SO ₂	NO ₂ /SO ₂	NO ₂ /O ₃	NO ₂ /O ₃ /SO ₂ MAK	NO ₂ /O ₃ /SO ₂ MIK	
PaO ₂	I	n	0	8	9	7	9	10	10	1
		p > 0.1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	> 0.1
	II	n	0	6	5	5	7	8	8	1
		p > 0.1	< 0.02	> 0.1	> 0.1	< 0.02	< 0.01	< 0.01	< 0.01	> 0.1
R _{aw}	I	n	2	8	7	4	9	7	9	1
		p > 0.1	< 0.01	< 0.01	> 0.1	< 0.01	< 0.01	< 0.01	< 0.01	> 0.1
	II	n	2	6	3	0	6	6	6	0
		p > 0.1	< 0.05	> 0.1	> 0.1	< 0.02	< 0.02	< 0.02	< 0.02	> 0.1

Table 1

Comparison of differences in PaO₂ and R_{aw} at the end of the 1 h prephase and the 2 h exposure phase by 2 different statistical approaches (I and II), II implying more stringent criteria.

I = PaO₂ decrease > 5 mmHg and R_{aw} increase > 0.5 cmH₂O/(l/s)

II = PaO₂ decrease > 5 mmHg and R_{aw} increase > 0.5 cmH₂O/(l/s) and outside the individual S.E. range

n = number of 'reacting' subjects

Increased reactivity becomes more pronounced after pre-exposure to the mixture of NO_2 , O_3 and SO_2 in MAK-concentration.

List of Publications Prepared Within the Contract:

NIEDING, G.von, WAGNER, H.M., LÖLLGEN, H., KREKELER, H.:
Zur akuten Wirkung von Ozon auf die Lungenfunktion des Menschen. - VDI-Berichte 270, 123-129 (1976)

NIEDING, G.von, WAGNER, H.M.:

Experimental Studies on the Short-term Effect of Air Pollutants on Pulmonary Function in Man: Two-hour Exposure to NO_2 , O_3 and SO_2 Alone and in Combination.
IVth Int. Clean Air Congress, Tokyo, May 1977
Proceedings: pp X 5 - 8 (1977)

NIEDING, G.von, WAGNER, M., KREKELER, H., BEUTHAN, A.,
FRIES, W.:

Bronchiale Hyperreagibilität nach Reizgasinhalation (NO_2 , O_3 und SO_2) in MAK-Wert-Konzentration.
17. Jahrestagung der 'Deutschen Gesellschaft für Arbeitsmedizin, Kiel, May 1977 - Jahresbericht: in press

NIEDING, G.von, KREKELER, H., LÖLLGEN, H., RIPLINGER, E.:

Intraindividuelle Variabilität von Lungenfunktionsgrößen im Längsschnitt und ihre Bedeutung für arbeitsmedizinische Untersuchungen. - Praxis Pneumologie: in press

NIEDING, G.von, WAGNER, H.M., KREKELER, H., LÖLLGEN, H.,
FRIES, W.:

Controlled Human Exposure Studies with the Air Pollutants NO_2 , O_3 and SO_2 Alone and in Combination.
Lung: in press

WAGNER, H.M., NIEDING, G.von, ANTWEILER, H., BEUTHAN, A.:

Biochemical Effects After Short-term Exposure of Humans to NO_2 , O_3 and SO_2 at MAC-Concentrations.
Int. J. Occ. Environ. Health : in press

SEIFERT, B., WAGNER, H.M., PRESS, H.:

Vergleich von Meßverfahren zur Bestimmung von Ozon.
Gesundheits-Ingenieur 97, 225 (1976)

Contractor: Techn. Universität München, Lehrstuhl für Tierzucht,
Freising-Weihenstephan, Deutschland

Contract no.: 015-74-1 ENV D

Project Leader: Dr. G. Stranzinger

Title of project: Chromosome analysis in the first division of early
embryos as a testmodel for environmental research.

Summary

Chromosome aberrations are the main cause for germ cell selection and embryonic mortality. Under certain conditions it is appropriate to use different experimental animals with a multiform chromosomal set. In addition to already existing testing systems, the developing embryo can be used for detailed in vitro studies concerning substances influencing the chromosomal make up. Unfortunately the preparation technique used on mouse embryos can not be transferred to rabbit embryos because of morphological differences.

The rabbit embryo culture from the one cell stage to the blastocyste stage can be successfully established with a medium worked out by MAURER (pers. comm.; medium is completely synthetic and not available commercially). Repeatable culture results with equal development of the blastomeres could be used for transplantations to ascertain the viability. Individuals out of transplantations have shown successful reproductive pattern with normal offsprings.

Adding known mutagenic compounds (Mitomycin C) and a compound in question (Clophen A 40) into the culture medium, the development of the embryos out of the one cell stage was blocked at the 4 cell stage. Control embryos developed to further cell stages. Different granulation of nucleus between

the different compounds could be seen.

Chromosome preparations are only successful with functional and dividing cells of embryos. The preparation technique for early rabbit embryos has been changed and we are using a 0.5 % trypsin solution as an enzymatic and hypotonic solution for removing the outer layers of the embryos and getting the blastomeres released for chromosome preparation. Pronase can not be used because of severe damage to the morphological make up of the chromosomes.

Division blockage is an additional parameter for certain effects of compounds on the embryo. Preparation success and blockage have to be used simultaneously for the interpretation of the action of compounds. It is interesting to note that no banding on the early embryonic chromosomes could be seen, even with prolonged and intense staining procedures. Morphological differences exist and may be able to be compared with results of drosophila chromosomes in the early embryonic divisions.

A total of 2830 embryos have been used for working out the preparation technique and comparing control and treatment group within the different experiments. Using the PCB Clophen A 40 in drinking water as the compound tested different amounts could be found in some organs and tissues respectively. The ovary showed a mean level of 0.1 ppm (Kidney 0.06 ppm - leg muscle 0.44 ppm - control 0.00 ppm). Ovulation rate and fertilization was not changed significantly but morphological differences on the ovary and embryo development could be seen.

Using the different techniques of embryo culture in a closed system, certain modifications and new techniques had to be worked out to make the system functioning. The very sensible system is to some extent complicated to standardize but when adapted to the laboratory conditions and personal a reasonable method to investigate environmental influences on embryonic development.

Publication:

Results of superovulation in two rabbit strains induced by a single PMSG-application.

Zuchthyg. 12: 14-18 (1977).

Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie,
D - 5948 Schmallenberg-Grafschaft

Contract n^o 017-74-1 ENV.D, Project No. 1

Project Leader: D. Kuhnen-Clausen

Title of project: Combined Action of Microconcentrations of
Detergents and Heavy Metals on Simple Biological Systems

The study was designed in order to examine the mutual interactions of detergents and heavy metals on biological systems in an aqueous environment. The assumption was made that water pollutants in concentrations at which each compound alone displays no or only weak toxic effects may produce synergistic or super-additive damage when they are simultaneously present. Isolated longitudinal muscles of the guinea pig ileum served as a model of a simple biological system exposed to environmental contamination. The alterations of its responses to cholinergic or histaminic stimulation can be used as a standard for different toxic effects.

The investigations dealt with the toxic effects of equimolar concentrations of either detergents or heavy metal salts with regard to the spasmogenic action of acetyl- β -methylcholine (MeCh) or, in some cases, of histamine. The detergent and salt concentrations ranged from 1 to 350 μ M and 0.1 to 10 μ M, respectively. The cationic N-dodecyl(=lauryl)pyridiniumchloride (LPC) and the anionic Sodium dodecylsulfate (SDS) served as model substances for detergents. The heavy metals lead, cadmium and mercury were used as chloride salts throughout.

The pharmacological experiments were carried out with the ileal muscle preparation (IMP), suspended in Tyrode's solution at 37^o C and pH 7.4, gassed with 5 % CO₂ in O₂. Isotonic contractions were recorded on a kymograph.

The tissue uptake of lead was measured as a function of the

incubation time by means of atomic absorption spectrometry. The result was compared with the spasmogenic effect of a longtime exposure of the tissue to lead.

Cumulative dose response curves to MeCh or histamine were recorded before and after the incubation of the IMP for ten min with either the detergents or the heavy metals. The test curves for the toxic effects of LPC or SDS were established in the detergent free suspension solution. Both compounds differ clearly with respect to their toxicity level and their mechanism of action. The incubation with $3.5 \mu\text{M}$ of LPC does not affect the action of MeCh or histamine. A tenfold higher dose of LPC, however, reduces the contractions' height induced by the spasmogenes. This effect is accentuated with each subsequent run of the dose response curves. $350 \mu\text{M}$ of SDS are needed to generate the doubling of the dose of MeCh in order to obtain half maximum contraction of the IMP. Because SDS does not affect the maximum response to MeCh it is considered to interfere with the binding of MeCh to its receptor protein. In contrast, LPC apparently impairs those processes which regulate the muscle contraction properly. The toxic effects of both detergents are irreversible within the experimental time.

At $0.1 \mu\text{M}$, PbCl_2 and CdCl_2 are weak inhibitors of the action of MeCh while HgCl_2 displays no effect. After increasing the salt concentration to $10 \mu\text{M}$, CdCl_2 and HgCl_2 show strong inhibitory effects which cannot be reversed by rinsing the IMP. $10 \mu\text{M}$ PbCl_2 causes either feeble inhibition or feeble acceleration of the effects induced by small concentrations of MeCh. These effects disappear after rinsing the test organ.

Against histamine, $10 \mu\text{M}$ of both PbCl_2 and CdCl_2 display inhibitory effects. However, the effect of PbCl_2 is reversible but that of CdCl_2 is irreversible and more pronounced.

The exposure of IMP to $10 \mu\text{M}$ PbCl_2 causes a slowly increasing contraction with a maximum obtained within one hour. Rinsing the preparation with lead free bath solution reduces the contraction to half of its maximum height within five minutes. The uptake and the release of lead, measured by means of atomic absorption

spectrometry, followed the same time course, showing that half of lead taken up is strongly bound to the tissue.

The toxicity of mercury compounds is assumed to be dependent on their high affinity for reactive -SH groups. A comparison of the action of HgCl_2 against MeCh with that of p-chloromercuribenzoate (PCMB) showed that the inorganic salt is more toxic than the organic compound. The thiol group reagent dithioerythritol (DTE) hinders or reverses the inhibitory effect of $15 \mu\text{M}$ PCMB completely but not the effect of $3 \mu\text{M}$ HgCl_2 .

The question arises, whether mercury compounds act exclusively on the cell surface or on intracellular compartments as well. The bispyridinium-dioxime TMB-4 is a muscarinic antagonist which cannot pass the cell membrane barrier because of its quaternary nitrogens. 0.1 mM TMB-4 was used in order to protect the IMP against PCMB or HgCl_2 . MeCh served as the agonist. Thin layer chromatography showed that no reaction product has to be taken into account when TMB-4 and PCMB are simultaneously present in the organ bath. TMB-4 produces a transient protection against PCMB but not against HgCl_2 . It is suggested, therefore, that PCMB displays its toxic effect on the cholinergic receptor on the cell surface while HgCl_2 reacts with -SH groups on the cell surface as well as on intracellular compartments.

The anticholinergic activity of LPC is possibly due to an overall induced disorder of the cell membrane structure and permeability. These effects may occur even at small concentrations of LPC which do not produce direct toxic effects and may influence the toxicity of heavy metal salts. A treatment of the IMPC with $1 \mu\text{M}$ LPC, after which no distinct toxic effect could be noted, increases indeed the very weak inhibitory effect of $1 \mu\text{M}$ of HgCl_2 against MeCh. However, the small activating or inhibitory effect of $10 \mu\text{M}$ PbCl_2 remained unchanged.

The results of this study illustrate that the toxic effects of detergents or of heavy metal salts can be demonstrated by the use of the IMP as a simple biological model. The toxic effects of water-contaminating poisons can be investigated qualitatively and quantitatively at concentrations corresponding to those found in fresh or waste water. The example of the enhanced toxicity of HgCl_2 after a pretreatment of the IMP with LPC shows that synergistic effects of water pollutants should be taken into account when they are simultaneously present.

Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie,
D - 5948 Schmalleberg-Grafschaft

Contract n^o 017-74-1 ENV.D, Project No. 2

Project Leader: K.-J. Hutter

Title of project: Investigations of Adverse Effects of Heavy-
Metal Compounds on Microorganisms by Means of
Pulse Cytophotometry

Pulse cytophotometry appears to be a suitable method for detection of cytotoxic effects because of its rapid measurement. The aim of the investigations was to develop a screening method which proves and characterizes the damage of heavy metal compounds on microorganisms.

Beside the protein content, the DNA-, RNA- and Chlorophyll-contents of microorganisms were examined. Beside pulse cytophotometric investigations single cell photometric determinations of the protein content of *Saccharomyces* yeasts were performed. In the course of the investigations it had to be clarified that with pulse cytophotometry the protein content of other microorganisms than *Saccharomyces* is detectable. Therefore yeasts of the species *Candida*, *Debariomyces*, *Hansenula*, *Kloeckera*, *Monilia*, *Rhodotorula*, *Schizosaccharomyces*, osmotolerant yeasts, bacteria of the species *Escherichia* and algae of the species *Chlorella* and *Scenedesmus* were included in the investigations. With reservations it can be concluded, that the protein content of microorganisms can be measured with pulse cytophotometry. The reservations apply to bacteria and populations which build mycelium or pseudomycelium, and grow in lumps, chains or budding bindings, respectively.

The influence of heavy metals on microorganism populations investigated after addition of lead acetate in concentrations of 2500, 1250, 100, 50 and 0,1 ppm Pb⁺⁺ and cadmium chloride in concentrations of 250, 125, 100, 90, 70, 50, 30, 10, 1, 0,1, and 0,01 ppm Cd⁺⁺.

The following results were found:

The addition of lead acetate in concentrations of 2500, 1250, 100 ppm Pb^{++} caused a decrease in the protein content of *Saccharomyces cerevisiae*. With pulse cytophotometry such changes can be registered by histograms within a few minutes.

The intensity of the fluorescence light signals due to the protein content of yeasts, was lower after lead intoxication. There was no change of the protein content of these cells after contamination with 50 and 0,1 ppm Pb^{++} respectively. Cadmium proved to be much more toxic than lead. Low concentrations of 250 to 1 ppm Cd^{++} produced already changes in protein content. There was no change at cadmium intoxication of 0,1 and 0,01 ppm Cd^{++} respectively.

Cadmium appears to alter the protein content of yeast cultures in concentrations as low as 1 ppm Cd^{++} , whereas lead caused damages occur in concentrations of more than 50 ppm Pb^{++} .

This result, found in *Saccharomyces cerevisiae*, cannot be transferred to other microorganisms. After addition of 4 ppm Pb^{++} and Cd^{++} respectively there was a change in Chlorophyll-content of algae as compared to the control suspension.

These heavy metal compounds result in a bleach out of algae, i.e. a smaller fluorescence intensity due to decreased Chlorophyll content.

Investigations in viable cells are a further step to use pulse cytophotometry in order to detect damages of microorganism following heavy metal intoxication.

Furthermore the changes of the protein- and Chlorophyll-content after addition of heavy metals were confirmed in single cell photometric measurements of the DNA content.

Attempts were made to measure DNA- and RNA-content with pulse cytophotometry but were not reproducible because of the small fluorescence intensity of these cell substances.

Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie,
D - 5948 Schmallenberg-Grafschaft

Contract n^o 017-74-1 ENV.D, Project No. 3

Project Leader: H. Muhle

Title of project: The Effect of Heavy Metal Inhalation on Cell
Number and Metabolism of Alveolar Macrophages
of the Mammal Lung - Lead and Cadmium -

Alveolar macrophages are characterized by their phagocytic properties particularly in relation to anti-microbial defense and alveolar clearance of inhaled particles. This study evaluates the effect of particulate matter containing heavy metals on the cell number and metabolism of mammal lung macrophages.

Groups of 12 to 20 male rats were continuously exposed to a PbCl₂ or CdCl₂ aerosol which was generated by an ultrasonic nebulizer. Specially constructed inhalation chambers guaranteed a homogeneous exposure of the rats to the aerosol. Another group of rats were used as controls breathing filtered air. Table 1 gives a summary of the inhalation experiments.

Expt. No.	Aerosol	Heavy Metal Concentration of the Aerosol (mg/m ³)	Duration of Exposure (days)	Median and Standard Deviation of the Aerodynamic Diameter (µm)
1	PbCl ₂	0,98 ± 0,18	91	0,61 ± 0,33
2	PbCl ₂	1,05 ± 0,13	21	0,61 ± 0,33
3	PbCl ₂	1,05 ± 0,13	7	0,61 ± 0,33
4	PbCl ₂	0,12 ± 0,03	91	0,33 ± 0,16
5	CdCl ₂	0,22 ± 0,06	66	0,32 ± 0,17
6	CdCl ₂	0,17 ± 0,05	7	0,32 ± 0,17
7	CdCl ₂	0,048 ± 0,015	7	0,32 ± 0,17

Table 1: Summary of inhalation experiments. The geometric standard deviation of the aerosol diameter is 1.7.

At the end of the exposure the rats were killed and the alveolar macrophages were isolated by standard pulmonary lavage. Body weight, lead or cadmium uptake of various organs, number of alveolar macrophages, their phagocytic activity and some histochemical and biochemical parameters were investigated and compared with the controls.

Statistical differences were estimated by the student t-test or the χ^2 -test (for the rate of mortality). If a result is significant, the error probability is given in brackets.

Results of lead exposure

1. In comparison to the controls a decrease in the number of alveolar macrophages washed out was found, e.g. Expt. No. 1: 35 % ($p < 0,002$); Expt. No. 4: 10 %.
2. At the same time, the volume of macrophages isolated from exposed animals had increased between 36 % (Expt. No. 4, $p < 0,001$) and 200 % (Expt. No. 1, $p < 0,001$).
3. The protein content per macrophage was enhanced e.g. 50 % (Expt. No. 4, $p < 0,05$) and 150 % (Expt. No. 1, $p < 0,001$).
4. For the quantitative investigation of the phagocytic activity of in vitro cultured macrophages a new method was developed. Fixed E.coli bacteria were stained with dansylchloride and given to the culture. The amount of incorporated bacteria was determined in a fluorescence microscope photometer. The phagocytic activity of macrophages isolated from exposed animals exceeded the controls from 100 % (Expt. No. 4, $p < 0,005$) to 300 % (Expt. No. 1, $p < 0,005$).
5. Two typical lysosomal enzymes were tested: β -glucuronidase and acid phosphatase. The macrophages from exposed animals showed increases of activity for both enzymes between 50 % (Expt. No. 4, $p < 0,05$) and 200 % (Expt. No. 1, $p < 0,001$).
6. With regard to changes in energy supply, the macrophages showed an increased pyruvate kinase activity, i.e. an enhancement of their glycolytic capacity ($p < 0,001$).
7. Histochemically an increase of acid phosphatase could be demonstrated.

Results of cadmium exposure

- Seven out of 20 rats died within 55 to 66 days after the beginning of an exposure to CdCl₂ aerosols (Expt. No. 5, $p < 0,005$).
- Body weight: a significant reduction ($p < 0,001$) was found in Expt. No. 5.
Control: 368 ± 30 g, $n = 20$, Cd exposed rats: 311 ± 50 g, $n = 13$.
- Lung weight: a significant increase over the controls was observed in two of three experiments, see Table 2.

Table 2: Changes of lung weight after CdCl₂ exposures

Expt. No.	Lung Weight			
	Control (g wet weight)	n	Cd-exposed (g wet weight)	n
5	$1,09 \pm 0,05$	10	$2,96 \pm 0,51^*$	10
6	$0,94 \pm 0,04$	6	$1,24 \pm 0,09^*$	6

*significant ($p < 0,001$)

- The lungs of exposed animals showed emphysemata associated with inflammatory changes (Expt. No. 5).
- Inflammatory changes are followed by an infiltration of macrophages, granulocytes and lymphocytes which are simultaneously obtained in pulmonary lavages. The content of macrophages within this mixture was about 50 to 70 %.
- In contrast to PbCl₂, CdCl₂ the number of alveolar macrophages increased by 30 and 70 % in Expt's No. 5 and 6. Macrophages were counted separately ($p < 0,001$).
- In all three experimental series, the volume of the macrophages was enlarged by some 100 to 180 % ($p < 0,001$).
- The phagocytic activity of the macrophages was enhanced by the CdCl₂ aerosol.
Expt. No. 5: 490 %, Expt. No. 6: 275 %, Expt. No. 7: 250 % ($p < 0,001$).

9. Histochemically, a strong increase in acid phosphatase activity was found for the alveolar macrophages of exposed animals. On the other hand, the β -glucuronidase showed little or no enhancement of its activity.
10. By electron microscopy, the content of lysosomes in the macrophages after CdCl_2 aerosol treatment was found to be much higher than in the controls. With increasing exposure time an increased number of vacuoles as well as electron-dense osmophilic myelin bodies was observed.

The fraction of binucleated macrophages was enlarged and a strong decrease of the number of mitochondria was observed. The remaining mitochondria were substantially enlarged and the matrix and cristae were considerably swollen.

Discussion

The results indicate that the enzyme activity and the phagocytic activity of alveolar macrophages are increased by the inhalation of lead or cadmium aerosols. This activation is possibly an unspecific effect of the inhaled irritant and could be an adaptation. Cadmium aerosol is more effective than lead, it causes a higher activation of the alveolar macrophages and an increased infiltration of granulocytes in the lung.

So far, the experimental evidence does not indicate whether and to which extent this adaptation enables the macrophages to prevent possible toxic effects of the inhaled heavy metals.

Alveolar macrophages seem to be a very sensitive parameter for investigations in pulmonary toxicology.

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The effect of chronic inhalation of lead aerosols on rat alveolar macrophages.

In press.

Auftragsnehmer: Johann Wolfgang Goethe-Universität,
Frankfurt am Main

Vertrag Nr.: 020-74-1 ENVD

Projektleiter : Dozent Dr. Jürgen Kleinebrecht

Titel des Projekts:

Teratogene und/oder mutagene Wirkung von
Schadstoffen.

Die Untersuchungen dienten dem Ziel, die zeitliche Abfolge mutagener und teratogener Veränderungen in Embryonen der Maus festzustellen. Als wirksames Agens wurde 5-Bromdesoxyuridin ausgewählt, dessen Teratogenität und Mutagenität erwiesen ist.

Die teratogene Wirkung von BUdR wurde am Tag IX der Schwangerschaft getestet. Im Bereich von 300 - 1200 mg/kg Körpergewicht konnten Dosis-Wirkungs-Beziehungen festgestellt werden. Bei der höchsten Dosis waren alle geburtsreifen Feten mißgebildet. Auffällig war, daß sich zwei Gruppen von Mißbildungen bilden ließen. Die eine Gruppe besteht aus Mißbildungen, bei denen zwar die Häufigkeit, aber nicht der Schweregrad mit zunehmender Dosis anstieg (z.B. Mikrophthalmie, präaxiale Polydaktylie). Bei der zweiten Gruppe (Wirbelsäule) nahmen sowohl Häufigkeit als auch Schweregrad mit steigender Dosis zu; hier gelten also die teratologischen Gesetzmäßigkeiten. Darüberhinaus bestand eine gewisse Organspezifität.

Nach Applikation von 1200 mg/kg BUdR am Tag IX der Schwangerschaft wurden Veränderungen der Mitoserate und der Rate an Chromosomenaberrationen untersucht (Tab. 1).

Im untersuchten Zeitraum 3 - 24 Stunden nach der Applikation ist in ganzen Embryonen sowohl bei den Kontrollen als auch in der BUdR-Serie ein Maximum der Mitoserate bei 6 Stunden zu verzeichnen. Nach 3 - 12 Stunden ist aber die Mitoserate in ganzen Embryonen der BUdR-Serie um 14-27 % niedriger als in den Kontrollen.

Nach 24 Stunden läßt sich kein Unterschied feststellen. In histologischen Schnitten wurde darüberhinaus die Mitoserate von Neuralgewebe (hohe Mitoserate), Somiten (mittlere Mitoserate) und Darm (niedrige Mitoserate) verfolgt. Wie in ganzen Embryonen war auch in den untersuchten Organen nach 3 Stunden die Mitoserate der BUdR-Serie um ca. 20 % niedriger als in den Kontrollen. Nach 6 Stunden war jedoch in Somiten und Darm eine vollständige Erholung eingetreten. Nur im Neuralgewebe war noch eine geringfügige Reduktion (6 %) vorhanden. Somit kann keines dieser Organe die im ganzen Embryo beobachtete stärkere Reduktion der Mitoserate bewirkt haben. Hier werden noch andere, stark proliferierende Organe untersucht werden müssen. Auch sollen Untersuchungen auf späteren Entwicklungsstadien angeschlossen werden.

Chromosomenaberrationen wurden in ganzen Embryonen im Zeitraum 3-24 Stunden nach der Applikation von BUdR (1200 mg/kg) am Tag IX der Schwangerschaft untersucht (Tab. 2). Schon nach drei Stunden waren erhöhte Raten von Gaps, Brüchen und Austauschfiguren festzustellen. Die Rate der Chromosomenaberrationen blieb im gesamten bisher untersuchten Zeitraum annähernd gleich.

Am Knochenmark adulter Mäuse wurden Dosis-Wirkungs-Beziehungen von durch BUdR erzeugten Chromosomenaberrationen untersucht (Tab. 3). Im untersuchten Zeitraum von 6-48 Stunden nach der Applikation war die höchste Rate von Chromosomenaberrationen nach 24 Stunden zu verzeichnen; dies gilt für alle Gruppen: Gaps, Brüche und Austauschfiguren. Zu diesem Zeitpunkt und zusätzlich weniger deutlich bei 48 Stunden wurden auch Dosis-Wirkungs-Beziehungen festgestellt.

Ähnliche Untersuchungen wurden auch für 2 weitere chemische Verbindungen gestartet: 1. Cyclophosphamid, 2. Methylmethansulfonat.

Die Auswertung dieser Untersuchungen konnte bisher nicht abgeschlossen werden. Die Ergebnisse müssen deshalb späteren Publikationen vorbehalten bleiben.

Tab. 1 Mitoseraten in Embryonen (n / 1000)

		ganze Embryonen	Neural- gewebe	Somiten	Darm
IX + 3 h	Kontrolle	36,5	22,5	14,2	4,7
	BUdR	31,4	17,5	11,4	3,7
IX + 6 h	Kontrolle	46,5	27,6	13,9	4,6
	BUdR	38,4	25,9	13,9	4,6
IX + 12h	Kontrolle	36,0	- ?	- ?	- ?
	BUdR	26,1	- ?	- ?	- ?
IX + 24h	Kontrolle	33,7	- ?	- ?	- ?
	BUdR	33,8	- ?	- ?	- ?

Die Raten in einzelnen Organen sind nicht mit der Rate in ganzen Embryonen vergleichbar, da unterschiedliche Bezugssysteme angewandt wurden.

? = Auswertung noch nicht abgeschlossen.

Tab. 2 Chromosomenaberrationen in Embryonen (%)

		Gaps	Brüche	Austausch- figuren
IX + 3 h	Kontrolle	0,9	0	0
	BUdR	4,3	3,0	2,0
IX + 6 h	Kontrolle	0,6	0,3	0
	BUdR	2,3	2,6	1,6
IX + 12h	Kontrolle	0,3	0	0
	BUdR	2,9	2,7	2,7

Tab. 3 Chromosomenaberrationen im Knochenmark (%)

Zeit	Dosis (mg/kg)	Gaps	Brüche	Austausch- figuren
	Kontrolle	1,1	0,4	0
6 h	600	1,3	1,3	0
	900	1,3	3,3	0
	1200	2,0	0,7	0
24 h	600	5,0	3,0	0,3
	900	3,0	7,7	0,3
	1200	8,0	13,4	4,8
48 h	600	3,2	3,3	0
	900	2,7	4,7	0
	1200	5,7	2,3	1,3

Contractor : Commission of the European Communities (Environmental Research
Contract No.: O21-74-1ENV D Programme)

Project Leader : Prof. Dr. Ulrich Mohr

Title of project : "Influence of micropollutants on the early development of
the sea urchin embryo"

The young sea urchin embryo (i.e. the fertilized egg through to the pluteus larva) represents not only an interesting system for embryology, but is also of general application for research into cell biology. The early developmental stages can be regarded as a special type of cell culture offering certain advantages over the classical mammalian cell cultures: natural synchrony of cell cleavage is observed in these early stages, cell cleavage as well as sequential differentiation processes can be investigated on both the morphological and molecular level and finally development proceeds in pure sea water. It is important in toxicological studies that the biological probe used is permeable to the applied substances. In contrast to other systems such as amphibian embryos, which are often used in basic research, the permeability of the sea urchin egg is increased after fertilization and non-ionic substances penetrate the embryos in the same manner as the often investigated mammalian cells. These advantages, in particular that of the morphological "diversity" observable during its early development, suggested the application of the sea urchin embryo as a model for the general toxicity of compounds discussed as micropollutants.

Technical details of the experiments are fully described in the listed publications (1-6). In brief, the main procedure consisted of presolving in dimethylsulfoxide the water insoluble substances and subsequently their slow addition to sea water to yield a fine and stable emulsion. DMSO in the applied concentrations was not toxic. The substances were added to different developmental stages and the effects were registered by microphotography. In most experiments, embryos from the species Sphaerechinus granularis and Paracentrotus lividus were used. One aim of the investigation was to compare the action of chemically related micropollutants.

Pesticides: Aldrin, dieldrin, endrin, heptachlor, DDT and methoxychlor were tested. The experiments are described in (3). Applied in concentrations of 0.02 mM/l dieldrin only did not affect development. The toxic activities according to influence on cell cleavage and differentiation can be ranked as follows: dieldrin < endrin < DDT < aldrin < heptachlor < methoxychlor. In contrast to findings in mammals, methoxychlor was more toxic than DDT in the sea urchin embryos; this indicates certain practical consequences for ecology that are more fully discussed in (3). Dichlorvos, an organophospho-insecticide, was tested separately; the substance acted similarly to DDT.

Carcinogens: The nitroso compounds N-nitroso-dimethyl-(DMN), diethyl-(DEN), dipropyl-(DPN), and dibutyl-(DBN) were tested (2), as well as N-methyl-N-nitrosourea (MNU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The toxicities of the nitrosamines increased logarithmically with respect to the number of C-atoms present (DMN 10^{-3} M). The absence of any measurable metabolic activity suggested the toxic action to be due to the substance itself. The higher toxicities of NMU (10^{-4} M) and MNNG (10^{-5} M) observed when these substances were tested under similar conditions to the nitrosamines is tentatively interpreted as being caused by reactive decomposition products. These two compounds are very unstable in water.

Aflatoxin B1: Continuous treatment from the two-cell stage in the presence of 10^{-5} M led to abnormal plutei. 10^{-6} M affected development only slightly. If added for only 30 min. at the two-cell stage, 10^{-5} M were less harmful than after continuous treatment. Nevertheless, the plutei were still abnormal.

Surfactants: Non-ionic surfactants of the Tween series were tested (4). No difference in activity was observed between Tween 20, 40, 60 and 80. In concentrations of 0.002%, development proceeded essentially undisturbed. Higher concentrations caused abnormalities. Concentrations of 0.01% (10^{-4} M) not only blocked cleavage in the two-cell stage (to which the compounds were added) but also lysed the embryos. In contrast to the toxic effects demonstrated by the other substances tested, the tweens caused special effects such as fusion of the embryos (0.004%) or exogastrulation (0.008%).

Derivatives of phorbol: Although these substances are not regarded as micropollutants, they still play an important role in cancer research as cocarcinogens. The highly cocarcinogenic substance, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and the inactive phorbol derivatives 4-methoxy-12-O-tetradecanoyl-phorbol-13-acetate (MeTPA) and 12-O-acetyl-phorbol-13-acetate (PDA) were tested. When permanently present from the two-cell stage to the pluteus larva, TPA-concentrations of 10^{-5} to 10^{-7} M led to a complete lysis of the two-cell stages. Furthermore, no cell cleavage was observed. Concentrations $<10^{-7}$ M allowed cell cleavage, while differentiation proceeded abnormally in the presence of concentrations of $>10^{-10}$ M. When present for 30 min. only normal plutei resulted below concentrations of 10^{-8} M. MeTPA was at least a hundredfold less active than TPA, while PDA did not influence development at all in concentrations $<10^{-5}$ M. For further data see (5). The results are of interest mainly for two reasons. Firstly, no other substance has been

reported as influencing development at such a low concentration as seen for TPA. Secondly, the low toxicities of MeTPA and PDA mirror their low activities as cocarcinogens. This result renders it likely that the mechanism by which the substances act as cocarcinogens might be closely related to the mechanism by which they act on the development of the sea urchin embryo.

All presented results have been described only briefly and for practical purposes morphological results only have been mentioned. Such morphological data are of major importance if the sea urchin embryo is to be applied as a test system for environmental studies. In addition to the described results on morphology, thymidine incorporation was measured as an indicator for DNA synthesis (1-6). Furthermore, mitochondrial densities were determined in untreated embryos (6). This experiment was planned not only for theoretical reasons, but was also necessary as a prerequisite for observing effects of micropollutants on micromorphology. Further studies in this direction are planned.

Final comments to the meaning of the sea urchin embryo as a test for micropollutants.

- 1.) Microsomal enzymes are not active in the sea urchin embryo. If not decomposed by other enzymatic systems, substances thus act directly. The toxicity of "inert" organic substances (= substances which do not demonstrate a specific toxicity) obviously depends upon the distribution coefficient of the substances in a hydrophilic-hydrophobic two phase system. According to the results described here and also to data published elsewhere, the limits of toxicities of water insoluble substances with molecular weights ranging between 260-600 rank in the order of 10^{-5} to 10^{-6} M.
- 2.) This unspecific toxicity is superimposed by a more specific action of certain substances as best demonstrated by the examples TPA-MeTPA, PDA and by methoxychlor-DDT.
- 3.) If compared to certain mammalian cell lines, in which microsomal activity is also low (e.g. the HeLa cell), the sea urchin embryo model represents in general a more sensitive indicator of toxic activities. This is since its differentiation processes not only provide greater information than is the case for simple cell cleavage in other systems, but also are more sensitive to lower concentrations of toxic agents. This has been demonstrated for the examined pesticides (unpublished results), TPA (5), and has also been discussed for the Tween-surfactants (4).

Practical consequences of the tests: Water insoluble organic substances (Mol. wt. 200-600) might be toxic not only for sea urchin embryos in concentrations $> 10^{-6}$ M, but also for other similar organisms, as for example

fish embryos. If this were the case, concentrations of such compounds should never be permitted to reach levels above 10^{-7} M in local river networks.

When human safety is considered, test systems offering a high microsomal activity are nowadays regarded as being of prior importance. However, for ecological reasons the actions of substances on other organisms have to be taken into consideration. A substance not toxic for man because of its quick metabolic elimination could nevertheless be toxic for other species not endowed with the metabolic activity necessary for elimination of the agent. The sea urchin embryo is a highly valuable tool for the latter type of test.

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We thank Christine Murphy for translation of the manuscript and Julia Dunning for secretarial assistance.

Contractor: Prof.Dr.Dr.H.Marquardt
 Forstbotanisches Institut
 Universität Freiburg

Contract n^o O22 - 74 -1ENVD

Project Leader: Prof.Dr.Dr.H.Marquardt

Titel of project: "Mutagenicity tests with automobil
 exhaust condensates in somatic cells".

A pattern of different genetic and cytogenetic tests has been developed with the aim:

- 1) to obtain reliable genetic data of a test compound,
- 2) to accumulate further data on the correlation between mutagenic and carcinogenic properties of a compound,
- 3) to apply a selected combination of tests in order to draw first conclusions on pharmacokinetics.

This test pattern covers both the molecular level and the chromosomal level.

Methods:

As a genetical system on the molecular level we used the induction of mitotic gene conversion in yeast cells (*Saccharomyces cerevisiae* D₄-RDII). Mitotic gene conversion is a mitotic recombination process which is closely correlated to mutagenicity. This recombination process is used in 4 different assays:

- 1) Direct treatment of the yeast cells with the test compound without activation.
- 2) Liver microsome test, with phenobarbital- and methylcholanthrene-induced microsomal fractions to activate the test compounds.
- 3) Host mediated assay, in which the yeast cells are injected in the ventral cavity of treated animals. The test compound is given per os.
- 4) Urinary assay: The urine of the first 4 hours of treated animals is added to the yeast cells, to detect excreted

metabolites. The test compound is given per os or intraperitoneally.

On the chromosomal level the induction of chromosome aberrations in the bone marrow of Chinese hamsters was used as a basis of the investigations. Since this test is very time consuming, a new cytogenetic in vivo test for the detection of sister chromatid exchanges (SCE) was developed as a sensitive and quick test.

Test Compounds and Results

As test substances we have chosen 2 carcinogenic cyclic hydrocarbons, 7,12-Dimethylbenzanthracene (DMBA) and 3,4-Benzo(a)pyrene (BaP) and 1 non-carcinogenic hydrocarbon Phenanthrene (Ph). Additionally N-Nitrosodiethylamine (DEN) and Methylmethansulphonate (MMS) were tested with the SCE method. Procarbazine (Natulan) was tested on the molecular and the chromosomal level.

The results with the hydrocarbons are listed in table 1.

<u>Genetic tests:</u>	DMBA	BaP	Ph
(Mitotic gene conversion in <i>Saccharomyces cerevisiae</i>)			
1) Direct assay without activation	-	-	-
2) Liver microsome test	+	+	-
3) Host mediated assay	-	-	-
4) Urinary assay	n.t.	n.t.	n.t.

Cytogenetic tests:

(Bone marrow of Chinese hamsters)			
5) Chromosome aberrations	+	<u>+</u>	-
6) SCE in vivo	++	+	<u>+</u>

n.t. = not tested.

As these cyclic hydrocarbons have to be activated to become mutagenic, the direct assay was negative with all 3 hydrocarbons. The liver microsome test revealed the genetic activity of the carcinogens DMBA and BaP, whereas the non-carcinogen Ph was negative. The negative results with all 3 hydrocarbons in the host mediated assay are due to the low sensitivity of this test. In the cytogenetic test DMBA, with a comparable strong activity was positive in both tests. BaP showed as clear positive result only in the SCE test. Ph, which was negative in all other tests, showed a weak positive result with high doses in the SCE-test.

Procarbazine was negative in the direct assay and the liver microsome test, but showed a clear positive result in the cytogenetic SCE test. MMS, often used as a strongly alkylating reference substance showed a clear positive effect in the SCE test. DEN, an indirectly acting substance, revealed a weak positive effect in the SCE test, when used in high doses.

From these results we can draw the following conclusions concerning the in vivo SCE test:

- 1) It is more than tenfold more sensitive than the aberrations test.
- 2) Because of this high sensitivity 50 - 100 cells per dose are sufficient to get reliable results instead of 1000 cells per dose in the aberration test. The SCE test is a quick test.
- 3) As dose response curve and the lowest effective dose can be determined with a little amount of time and animals.

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Contractor: Medizinisches Institut für Lufthygiene und
Silikoseforschung an der Universität Düsseldorf

Contract n^o 061 - 74 - 1 ENVD, Project 1

Project leader: Prof. Dr. med. H.-W. Schlipkötter

Title of project: Histological and autoradiographic
investigations on the effects of lead,
cadmium, and mercury on the embryonic
organogenesis and germ cells and the
placenta

This work was carried out to investigate the teratogenic action of cadmium and especially to elucidate on which day of gestation cadmium is most toxic to the embryo or fetus. We had several series which will be described briefly.

1. Series

Pregnant rats were injected i.p. once with 2.5 mg CdCl₂/kg body-weight on the 7th to the 21st day of gestation. The fetuses were removed on the 22nd day of gestation. This concentration was lethal to a number of fetuses, others showed malformations occurring when cadmium was administered on the 7th, 8th and 10th to the 14th day of pregnancy. These malformations concerned the cranium (reduction of the thickness of the vault, dehiscent fontanelles), the ribs, the vertebrae and the sternum, and the eyes (anophthalmia and microphthalmia).

2. Series

Pregnant rats were injected i.p. once with 5.0 mg CdCl₂/kg body-weight. 24 hours after administration, they were sacrificed and the embryos were sectioned in series. No marked alterations were found in embryos whose mothers were injected on the 8th day of gestation, while after injection on the 9th day, the embryos showed several necrotic cells in

the epithelium of the embryoblast, for example. The extra-embryonal mesoderm was not that much affected. After injection on the 10th day several necrotic cells in the district of the neural tube but no other injuries were detected. Injection on the 11th day resulted in numerous necrotic cells in the region of the neural tube and its derivatives, the optic vesicles and the somites. The effects were wide-spread, though mitosis were still observed in the close surroundings. After injection on the 12th day of gestation the embryos did not seem to be that sensitive. The various fetal modifications of the several litters with the same dose of CdCl_2 cannot be explained alone by a different sensitivity on the various days of gestation. The biological factor has still to be elucidated.

3. Series

The methods of this series correspond to those of the 2nd series, but the rats were injected with 2.5 mg CdCl_2 /kg body-weight instead of 5.0 mg/kg. These investigations are not completed yet, so only a few results can be listed.

After injection on the 10th day of gestation one of three embryos was retarded, the other two were almost resorbed. Only necrotic giant cells of the trophoblast, cell detritus, necrosis of the decidua cells and coagulated blood in the lumina of the uterus were found after injection on the 11th day.

We have not clarified yet, if injection of 2.5 mg causes the same injuries to the neural tube and its derivatives as we described after application of 5.0 mg CdCl_2 . The anophthalmia and microphthalmia as well as the malformations of the skeletal system could be well explained by this finding. It would indicate that the necrotic cells in the area around the optic vesicles and the somites interrupt the continuity of the "cell line" and by this malformations of the eyes or

skeleton might occur.

Summary

A single i.p. injection of 2.5 mg CdCl₂/kg body-weight to pregnant rats caused injury to the skeleton and eyes on fetuses examined on the 22nd day of gestation. The "critical days of gestation" on which the somatic cells of the developing embryos were most sensitive to cadmium are days 7 to 14.

Histological studies from embryos after a single injection of 5.0 mg CdCl₂/kg showed that before development of the somites, the epithelium of the embryoblast is most sensitive, while after development of the somites, the somites, neural tube, and optic vesicles are most sensitive.

Publication

These results were presented on the meeting of "Deutsche Gesellschaft für Hygiene und Mikrobiologie", Mainz October 1976

Contractor: Medizinisches Institut für Lufthygiene und
Silikoseforschung an der Universität Düsseldorf

Contract n^o 061 - 74 - 1 ENVD, Project 3

Project leader: Prof. Dr. med. H.-W. Schlipkötter

Title of project: Cell culture as a test system for
analysis of the biological, especially
oncogenic effect of substances from
the air

City smog can induce a toxic effect on men, animals and plants. Using cell cultures as a test system it is possible to analyse toxic effect of city smog on cellular and molecularbiological level.

Mainly two effects caused by city smog are of special importance. First to mention the toxic effect of city smog exerted on mammalian cells in vitro. This toxic effect is responsible for alterations ranging from minimal disturbances of cellular function to cell death or necrosis.

It is important that this alterations can be reversible or irreversible in nature.

The second effect of city smog comprehends carcinogenic activity, which is important for transformation of normal cells to cancer cells. As carcinogenic transformation involves an alteration of the genetic material of the cell it is irreversible.

Analysis of cytotoxicity

In our experiments samples of city smog from the Ruhr-area in Federal Republic of Germany were extracted by acetone and DMSO or only DMSO respectively. The amount of benzo(a)pyren (BP) in extracts was used as a measure for standardization and dosage.

We employed macrophages of the mouse (line IC-21) for toxicity analysis of city smog extracts, because in humans also macrophages of the lung are target cells for air pollutants.

City smog extract No. 11 A,B was collected in Bochum from February 2 - 24, 1970, and extracted by acetone-DMSO (11 A) or only DMSO (11 B).

City smog extract No. 11 A,B in culture medium, containing a BP-equivalent of 0,1 µg/ml exerted no measurable alteration in our test system, however, a concentration of 1,0 µg/ml BP-equivalent produced a remarkable cell damage.

Viability of cells measured by dye exclusion test was rapidly lost after more than five hours incubation time in presence of city smog extract. 24 hr after incubation has started percentage of damaged cells amounted to more than 90 %.

We found no differences in toxicity of city smog extracts prepared by acetone-DMSO or DMSO only.

We also observed an increased release of lactate-dehydrogenase in the medium in presence of city smog extract.

This indicates that city smog extracts lead to an increased membrane permeability and damage.

Lactate production and glucose consumption reflect energy and carbohydrate metabolism of cells. Under influence of city smog extracts production of lactate in cultures of mouse macrophages was reduced.

As a further test system for analysing biological effects of city smog extracts we choose human lymphocytes from the peripheral blood.

As a sensitive parameter of cytotoxicity we employed the induction of DNA synthesis in lymphocytes by phytohemagglutinine. Rate of DNA synthesis was measured by

incorporation of ^3H -thymidine. Radioactivity of precipitable material was finally measured in a TriCarb-Scintillation counter.

Our results demonstrate a dose dependent inhibition of cellular DNA synthesis in lymphocyte cultures by city smog extract. This inhibition was especially pronounced using a concentration of $1\ \mu\text{g}/\text{ml}$ BP-equivalent. A remarkable reduction of DNA synthesis was also detected using $0,1\ \mu\text{g}/\text{ml}$ BP-equivalent.

It is interesting to mention that there exist a good agreement in detection of cytotoxicity of city smog extracts using mouse macrophages.

Analysis of carcinogenicity

According to experiments of Rhim and Huebner cell cultures infected with some viruses are a sensitive tool for detection of carcinogenic activity of environmental pollutants. Syrian hamster cells in vitro can be transformed to cancer cells by the DNA-containing Papovavirus Simian Virus (SV) 40. We used this assay for analysis of transforming activities of city smog extracts and of some components.

In our experiments we exposed syrian hamster kidney cells to city smog extract No. 11 B at a concentration of $0,01$ and $0,1\ \mu\text{g}/\text{ml}$ BP-equivalent for 18 hours. Thereafter cells were infected by SV40.

Combined treatment of hamster kidney cells with city smog extract and SV40 produced a striking increase of transformation frequency.

We observed a similar enhancement in transformation by pretreatment of cells with carcinogenic polycyclic hydrocarbons benzo(a)pyren of 7,12-dimethylbenzanthracene employing the same assay.

Publications

Seemayer, N., N. Manojlovic und A. Brockhaus:

Die Wirkung von Feinstaubextrakten auf Zellen in vitro unter besonderer Berücksichtigung krebserzeugender Komponenten. 35. Tagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie, Mannheim 28.9. - 1.10.1975

Seemayer, N., N. Manojlovic und A. Brockhaus:

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Seemayer, N., N. Manojlovic and N. de Rooter:

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Interaction of Chemical Carcinogens and the Papovavirus SV40 in Cell Transformation (Abstract). Xth Meeting of the European Tumour Virus Group, Grindelwald (Switzerland), October 3 - 7, 1976

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Untersuchung der biologischen Wirkung von atmosphärischen Feinstaubextrakten. I. Prüfung der zytotoxischen Wirkung eines Feinstaubextraktes aus einem Ballungsgebiet und von polyzyklischen Kohlenwasserstoffen an Mäusemakrophagen in vitro. Zbl. Bakt. Hyg., I. Abt. Orig. B, in press

Contractor: Medizinisches Institut für Lufthygiene und
Silikoseforschung an der Universität Düsseldorf

Contract n^o 061 - 74 - 1 ENVD, Project 2

Project leader: Prof. Dr. med. H.-W. Schlipköter

Title of project: The metabolism of benzo(a)pyrene in the
lung under the influence of air pollutants

The purpose of our study is to detect possible coergistic effects of different air pollutants. It is assumed that the carcinogenic effect of benzo(a)pyrene, which is a common air pollutant, might be influenced by other pollutants. It is widely accepted now, that benzo(a)pyrene like other polyaromatic hydrocarbons is metabolized by the mixed function oxidases to the ultimate carcinogen. Probably epoxides and hydroxymethyl derivatives are the carcinogenic metabolites. We assume that some air pollutants could alter the metabolic pathways of benzo(a)pyrene and thus influence the carcinogenicity of this compound.

Benzo(a)pyrene is found preferentially in the finer particles (smaller than 5 micrometers) of the airborne aerosols. It is deposited therefore in the bronchiolar and alveolar region of the lung. Hence we assume that benzo(a)pyrene is phagocytized for the most part by alveolar macrophages. This is the reason, why we are using this cells, which are obtained by lung lavage from guinea pigs.

As we have shown in our previous work, alveolar macrophages metabolize benzo(a)pyrene. The metabolites can be separated by chromatographic methods. We are using High Speed Liquid Chromatography now. A characteristic metabolic profile is thus obtained. Specific influences of pollutants on the metabolic pathways of benzo(a)pyrene are expected to alter this profile.

The pollutants, which have been under study until now, are lead and cadmium.

Experimental

(1) In vivo experiments:

Guinea pigs are kept in inhalation chambers. The animals are exposed to a lead aerosol for 8 hours per day (23,45 h - 7.45 h). The aerosol is generated from a solution of lead chloride. The lead concentration is measured by a filter technique and atomic absorption spectrometry.

The concentration of lead in the atmosphere of the inhalation chamber was $189 \pm 59 \mu\text{g}/\text{m}^3$.

Immediately after the exposure two guinea pigs are removed from the inhalation chamber. The animals are killed in narcosis and the alveolar macrophages are washed out of the lungs. The cells are resuspended after centrifugation and spread in plastic Petri-dishes.

Benzo(a)pyrene is added and after an incubation period of 4 hours the metabolites are extracted. The radioactivity remaining in the aqueous phase is measured by liquid scintillation counting. This measurement reveals the "water soluble metabolites". The organic extract is evaporated to dryness, solved in ethanol and chromatographed by High Pressure Liquid Chromatography. The detection is achieved by a radiochromatograph. The peaks are evaluated quantitatively by an integrator.

(2) In vitro experiments:

Alveolar macrophages are washed out of the lung of untreated guinea pigs as described. The cells are spread in glass Petri-dishes. Labelled benzo(a)pyrene (14-C) is added and

lead (10-30 mM) or cadmium (1-10 mM). After an incubation period of 4 hours the cells and the supernatant is extracted by ethylacetate, and after evaporation the extract is chromatographed by High Speed Liquid Chromatography on reversed phase columns. Samples (0,5 ml) are collected by a fraction collector, scintillation fluid is added and the radioactivity is measured.

Results

1. The exposition to lead in vivo reduces the number of alveolar macrophages, which can be obtained by lung lavage. On the average the number of cells is decreased to $82 \pm 9 \%$ if compared to control animals. This result is in good agreement with the study of Bingham, who described a 60 % decrease in the number of alveolar macrophages of rats exposed to air containing $10 \mu\text{g}/\text{m}^3$ lead during 12 months. In our experiments with higher lead concentrations this effect became evident already after some days.
2. The capability of alveolar macrophages of exposed animals to metabolize benzo(a)pyrene to water soluble metabolites is decreased, to $81 \pm 9 \%$ if compared to control caused by an inhibition of the synthesis of cytochrome P-450, which prevents the induction of the mixed function oxidases. This reduction in the amount of metabolites formed per cell is in agreement with our own results published previously.
3. The suspected alteration of the metabolic profile could not be demonstrated. There was no difference between exposed and control animals. The quantitative relation between two of the main metabolites, the 7,8- and 9,10-diols of benzo(a)pyrene was calculated from liquid scintillation counting data. A constant value of 1.8 ± 0.2 was obtained from chromatograms of both exposed and control animals.

4. The addition of lead in vitro to alveolar macrophages shows the same result. The metabolites produced by untreated and lead treated macrophages are the same, the metabolic profile is not changed. But the amount of metabolites produced per cell is reduced.

5. The addition of cadmium in vitro to alveolar macrophages has the same effect. The metabolic profile is compared with untreated cells unchanged, but the yield of metabolites is reduced.

Publication

These results were presented on the meeting of "Deutsche Gesellschaft für Hygiene und Mikrobiologie", Mainz October 1976.

Contractor : Gesellschaft für Strahlen- und Umweltforschung, Abt. für Genetik, Neuherberg, FRG

Contract No. : O66 - 74 - 1 ENV D

Project Leader : Dr. U.H. Ehling

Title of project : Chemically-Induced Mutations in Mice

The main scope of this project is a) the determination of dose-effect relationship for different kinds of mutational and chromosomal damage in mammals, b) the determination of the sensitivity of the different methods for selected compounds, c) investigation of the germ cell stage specific induction of chemically induced dominant lethals, chromosomal aberrations, and specific locus mutations.

Extensive experiments have been conducted using methyl methanesulfonate (MMS), Natulan[®], and mitomycin C as test compounds. The essential results are as follows:

Dominant Lethal Mutations

To determine the sensitivity of the dominant lethal assay (101xC3H)_{F₁} hybrid male mice were injected intraperitoneally with 1 ml of the test compound. The weights of animals ranged between 27-30 g and did not vary from the nominal value by more than 5%. Immediately after treatment each male was mated sequentially to one (101xC3H)_{F₁} virgin female in 4-day intervals up to 48 days. The age of the mice when first mated was 89-98 days. The uterine contents of females were examined 14-17 days after conception.

With a sample size of 10 males per dose group the induction of mutations with 40-150 mg/kg of MMS can be detected. Increasing the sample size to 45 males per dose group the

lowest effective concentration which induces dominant mutations is 10 mg/kg. This dose induces 6-11% dominant lethals in spermatids in the mating interval 9-12 days postinjection. In one experiment the mutations were mainly due to postimplantation loss ($P < 0.04$), in the other experiment the preimplantation loss was significantly increased ($P < 0.03$).

The induction of dominant lethal mutations in the range of 200 - 800 mg/kg of Natulan[®] and 1.75 - 7.0 mg/kg of mitomycin C can be detected with a sample size of 20 males per dose group. Experiments with a sample size of 45 males per dose group are in progress. With this sample size it is possible to detect the induction of mutations with 100 mg/kg of Natulan[®] and 0.75 mg/kg of mitomycin C.

Heritable Translocations

Heritable translocations were evaluated in the most sensitive stages of spermatogenesis to the induction of dominant lethal mutations by MMS, i.e. after treatment of spermatozoa and late spermatids. With 40 mg/kg of MMS the frequency of sterile and partially sterile F_1 progeny was 11.2%. All partially sterile and 6 of the 14 sterile F_1 males were cytogenetically demonstrated to be translocation carriers (8.0%). Transmission of the translocations was confirmed in sons of 13 partially sterile translocation heterozygotes by fertility testing as well as by cytogenetic analysis.

In vivo Cytogenetics

In experiments with Natulan[®] and mitomycin C chromatid aberrations were induced in differentiating spermatogonia. When treated spermatogonial stem cells were analysed after they had developed into primary spermatocytes, however, no translocations were observed with single treatments and only a none dose-dependent increase of translocations was found after repeated treatment. From both the translocation experiment and the in vivo cytogenetic studies it was concluded that germinal selection effectively selects against chromosomal

aberrations induced in premeiotic germ cells while little or no selection exists against aberrations induced in postmeiotic germ cells.

Specific Locus Mutations

The frequency of specific locus mutations after i.p. injection of 600 mg/kg of Natulán[®] in postspermatogonia is 14.8×10^{-5} mutations/locus/gamete and 5.0×10^{-5} mutations/locus/gamete in spermatogonia. Both mutation rates are highly significantly different from the control frequency of 0.8×10^{-5} mutations/locus/gamete.

A dose of 40 mg/kg of MMS induced in the mating intervals 5-12 days posttreatment 30.2×10^{-5} mutations/locus/gamete. However, MMS is ineffective in inducing mutations in spermatogonia. Because of the implications of this observation large-scale experiments with MMS are in progress to test the differential spermatogenic response for the induction of specific locus mutations by MMS.

In contrast to MMS, the mutation rate in postspermatogonia with mitomycin C is similar to the spontaneous rate. The frequency of 1.4×10^{-5} mutations/locus/gamete after treatment of postspermatogonial germ cell stages with 5.25 mg/kg of mitomycin C is not significantly different from the control frequency ($P=0.41$). The overall mutation frequency of the higher dose groups (5.25-7.00 mg/kg) is 4.7×10^{-5} per locus per gamete for spermatogonia. The difference between the overall mutation rate in these mitomycin C groups and the control group is significant ($P=0.02$).

Summary: Mutagenicity in Mice

Compound	Test Systems				Somatic Mutations in vivo	Specific Locus Mutations
	Dominant Lethals	Heritable Translocations	Chromosome aberrations Spermatocytes	Spermatogon.		
MMS	+	+	n.t.	n.t.	n.t.	+
Natulan [®]	+	n.t.	n.t.	+	+	+
Mitomycin C	+	n.t.	+	+	n.t.	+

n.t. = not tested

Completed studies funded under contract no. O66 - 74 - 1 ENV D

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Contractor: Justus-Liebig-Universität Giessen, Hygiene-Institut

Contract-No. 071-74-1 ENVD

Project-Leader Prof. Dr. med. Ernst Gerhard Beck

Title of project "Toxicological studies of environmental pollutants using cell cultures as test systems"

During the research period our efforts were mainly directed to studying the cytotoxicity of ambient dust and its components testing the total aerosol and, as components, heavy metals and fibrous dusts.

I. TOTAL AEROSOL

a) Results

Cytotoxicological investigations were made of ambient dusts collected at measuring stations located at Gelsenkirchen, Deuselbach, Schauinsland, Düsseldorf and Bochum. The samples were collected during January to April 1973. During the sampling period the median dust concentration at Gelsenkirchen was 3 - 8 times higher than at Deuselbach and Schauinsland which have little or no industry, and the benzo(a)pyrene concentration per gram dust was 11 - 12 times higher. In spite of the longer sampling periods (2 - 4 weeks) the samples from Deuselbach and Schauinsland contained less dust than those from Gelsenkirchen (duration of one sampling period 3 - 5 days). In order to have enough dust for the cytotoxicological studies, several samples were combined into a single sample. The samples collected within the same period of time at different measuring stations were membrane-pathogenic to a different degree when applying the same amounts of dust, regardless of the actual local concentrations of ambient dust. Until the 5th hour after exposure the samples collected at Gelsenkirchen and Bochum caused at 1.5-fold higher enzyme release in guinea pig macrophages in vitro than those from Düsseldorf, and a 3 - 5-fold higher release than those from Deuselbach and Schauinsland. According to morphological and cytochemical criteria 100 % of the cells incubated with samples from Gelsenkirchen, Bochum and

Düsseldorf were membrane-damaged in comparison to 65 % and 30 % of those from Deuselbach and Schauinsland.

b) Evaluation

It was the purpose of these studies to use the test system cell culture for the evaluation of the potential toxicity of immission from different localities and to elucidate their mechanisms of action. The results demonstrate that it is possible to qualitatively compare particulate immissions as regards their biological effects. However, the evaluation of the results following exposure of the cells to the different aerosols collected at the above-mentioned measuring stations was extremely difficult and problematical for the following reasons:

1. Different dust concentrations at the sampling stations require different sampling times. This could not be taken into account in our experiments.
2. The extent to which possible secondary alterations due to meteorological factors such as humidity occur on the sampling filters also depends on the length of the sampling period. Such alteration may again influence the toxicity of ambient dusts.
3. Furthermore, experimental evidence indicates that the toxicity of samples from woody locations may be produced by substances of plant origin, such as amines and terpenes. Thus the total toxicity of dusts samples can be caused by radically differing factors.

Because of the fundamental difficulties in the evaluation of the results obtained through the studies of cells exposed to total ambient aerosols, additional investigations were carried out using single components of ambient dust.

II. HEAVY METALS

a) Results

In short and long-term experiments the acute and chronic effects of heavy metals was studied using tissue culture cells in vitro. As test objects were used static suspension cultures of L-A-cells, a subline of L 929 mouse fibroblasts.

1. Cultivation of the cells with lead caused a dose-dependant inhibition of proliferation and cell death. The growth inhibition was independent of the anion used and could be prevented by the addition of Ca-EDTA. The LD_{50} was approximately 1 mM when exposing the cells for 2 days and 2×10^{-4} M when treating them for 7 days. The minimal growth inhibiting dose was in the range of 2 to 4×10^{-5} M. Concomitant with the dose-dependent inhibition of proliferation the fraction of cells synthesizing DNA and the mitotic rate were lowered, suggesting a block in the G1 phase of the cell cycle. Energy

metabolism appeared affected as evidenced by a rise in lactate production. Lactate dehydrogenase release was in the control range. Lead induced an increase in cell size and, at higher concentrations, cellular vacuolations. Under continuous exposure lead tolerance developed, even to concentrations exceeding to LD_{50} . The kinetics of the acquirement of resistance were demonstrated. The resistant cells adjusted more quickly to higher doses of the heavy metal than the controls. Low-resistance strains retained the growth characteristics of the parent cells, but with increasing levels of lead tolerance multiplication rates decreased significantly. After approximately 60 generations in lead-free environment the cells had to a major degree lost their resistance indicating that the induced lead tolerance is caused by adaptation rather than by mutation and deletion. Lead resistance appears not to depend on lead exclusion, for the lead content of resistant cells and lead-exposed controls was found to be of a comparable order of magnitude.

2. In further experiments the toxicity of the heavy metals mercury and cadmium was compared to that of lead. As regards lethality and proliferation inhibition an increasing cytotoxicity was noted from lead over mercury to cadmium. The LD_{50} following 7 days' exposure was 2×10^{-4} M for lead; 5×10^{-5} for mercury and $1,3 \times 10^{-5}$ M for cadmium. The doses causing a 50 % inhibition of increase in cell number (ID_{50}) after 7 days' application were determined as follows: lead, 4×10^{-5} M, mercury $1,3 \times 10^{-5}$ M, and cadmium, $7,5 \times 10^{-5}$ M. In long-term experiments it was observed that L-A-cells can also develop resistance against cadmium and mercury.

b) Evaluation

1. It is possible to establish a toxicological rank order of the heavy metals tested.
2. With the in vitro system statements can be made regarding the mechanisms of action of heavy metals at the cellular level.
3. Of practical relevance is the observation that the cells are capable of developing resistance against heavy metals. This phenomenon has also been observed in vivo. It should be of interest to know what are the conditions for the development of resistance, by which factors they can be influenced and to follow up the problem of cross-resistance.

III. FIBROUS AMBIENT DUST

a) Results

Ambient dusts from congested areas and cities may contain fiberform material. When testing biological effects of e.g. asbestos and glass fibres it was demonstrated that fibers shorter 5 μm have no acute cytotoxic effect, while fibres longer than approximately 10 μm and with a mean diameter of 0.5 μm are chronically membrane-pathogenic. Phagocytosis of long mineral fibres is delayed and remains partly incomplete. This results in a localized, increased permeability of the cells. A permanent loss of enzymes follows which is compensated for by an elevated glycolytic metabolism. In connection with the pathological effects of long mineral fibres on the cell membrane it is of interest that asbestos and glass fibres induce the formation of polynuclear giant cells by fusion. In the process of cell fusing asbestos can activate integrated virus genomes and release infectious viruses (collaboration with Prof. Seemayer and Dr. Manojlovic, Medizinisches Institut für Lufthygiene und Silikoseforschung, Düsseldorf).

b) Evaluation

1. The decisive factor for the biological effect of fibres is their shape (length, diameter), unless in addition their elasticity is of pathogenic importance.
2. The finding that long fibres can lead to the fusing of cells is of special interest. The resulting release of integrated virus genomes should be interpreted with caution, but should not be forgotten with regard to carcinogenesis caused by fibrous dusts. In this direction there are important possibilities for further research. The chronic interaction between cell and long mineral fibre is being discussed as a factor for the development of fibrosis and cancer. Should the final proof be established that the oncogenic effect of a fibre is limited by a minimal length and maximal diameter, the actual question would be closer to solution whether inorganic fibres in the ambient air constitute a health hazard for the normal population.

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Contractant : Institut National de Recherche Chimique Appliquée (IRCHA),
Vert-le-Petit, France

Contrat n° : 056-74-1 ENV F

Chefs de projet : M. R. Cabridenc, Prof. R. Truhaut

Titre du projet : Evaluation toxicologique des micropolluants
organiques susceptibles d'être présents dans
les eaux destinées à la consommation humaine

Le rapport final n'est pas encore disponible, l'évaluation
statistique des résultats étant en cours.

Contractant : Bureau de Recherches Géologiques et Minières
(J. GONI)
Laboratoire de Biopathologie Pulmonaire
(J. BIGNON)

N° du Contrat : 098 - 75 - 1 ENV F

Chef du projet : Pr Agr J. BIGNON - J. GONI

Titre du projet : Physico-chemical interactions of chrysotile with SO₂. Methodology for investigation of the asbestos toxicity. Study of the toxicity of natural chrysotile with respect to chrysotile + SO₂.

The aim of this work was to compare the toxic effect of natural UICC A chrysotile and chrysotile treated with SO₂, in order to determine a possible synergistic effect. Then, the first part was a physico-chemical study of the mechanisms of SO₂ adsorption on the fibers. In the second part, biological investigations were performed in vivo, in animal experimentation ; and in vitro, on alveolar macrophages and red blood cells.

I. The physico-chemical studies have included the observation of the adsorption of H₂O and SO₂ in dry and humid atmosphere using static and dynamic methods. Experiments were carried out with manual sorpmetre for static analysis and gas chromatography for dynamic analysis. This studies showed that, under the mean atmospheric conditions : hygrometry, partial pressure of SO₂, this gas was strongly chemisorbed. However, although the presence of SO₄ was shown by analysis with photo-electron spectroscopy, it was not possible to find out the true nature of bonding on asbestos fibers and the related consequences on physico-chemical properties of fibers and on sulphur mobility. Sulphur seemed to be relatively free, since SO₂-sorbed fibers released some sulphate when they were suspended in water. The samples given for biological studies were < 100 μm length and the amount of SO₂ sorbed was 4 ppm.

II. Biological studies have included in vitro and in vivo tests.

1. In vitro experiments have been carried out using red blood cells (RBC) and alveolar macrophages (AM) from rabbits. RBC were obtained by puncture on the ear marginal vein. The effect on RBC was demonstrated by the measurement of K^+ and hemoglobin release, in presence of chrysotile and SO_2 -chrysotile. For example, when 5 % suspension of RBC were mixed with both chrysotile, a 60 % hemolysis and 80 % K^+ release were observed at 120 min. The two kinds of chrysotile tested showed the same hemolytic capacity. AM were obtained by pulmonary lavage with 0.9 % NaCl. The study consisted in the measurement of the cell viability and release of enzymes from cells in culture. The toxic effect of UICC A chrysotile and SO_2 -chrysotile with regard to control without fiber, was deduced from a decrease (about 38 %) of the viability, and from the release of cytoplasmic (LDH) and lysosomal enzymes (β glucuronidase, N Ac β D glucosaminidase and β galactosidase). However, there was no difference between both type of chrysotile (UICC chrysotile and SO_2 -chrysotile).

2. Biochemical and cellular in vivo tests were carried out from rabbits receiving one intratracheal injection of both types of chrysotile, and sacrificed 68 hours after the injection. AM were then recovered by pulmonary lavage. The centrifugation (270 g 10 min) of the fluid lavage gave a supernatant processed for lipids and proteins analysis, and a cell pellet containing more than 95 % of AM.

The lipids and proteins analysis did not show any significant difference between the two groups of animals (UICC chrysotile and SO_2 -chrysotile). Moreover, in these experimental conditions, the alveolar biochemistry was not very different from the control group.

The enzymatic activities of alveolar macrophages from animals injected with SO_2 chrysotile showed a significant increase of the enzymes LDH and acid phosphatase. The LDH increase could be related to the affinity of the enzyme regarding some chemical forms of SO_2 . The other enzymatic activi-

ties were not modified.

3. Histologic studies have shown moderate focal lesions of interstitial pneumonitis and fibrosis. The lesions were more severe after 7 days than after 68 hours.

Thus, this study has shown that chrysotile fibers can adsorb SO_2 . The in vitro toxicity of UICC A chrysotile was confirmed. In contrast, under the experimental conditions used (intratracheal injection of a single dose, 68 hours lapse-time), the biochemical, biological and histological changes due to chrysotile were slight. There was no increase of the toxic effects of chrysotile after chemisorption of SO_2 on the fibers. This fact, controversial with other workers, might be due to the chemical form of sorbed SO_2 (sulphate).

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Contractant : Université Paul SABATIER. TOULOUSE FRANCE

N° du contrat : 103 - 75 - 1 ENV. F

Chef du projet : Professeur R. DERACHE

Titre du projet : Effets physio-pathologiques de l'étain contenu dans les aliments.

L'étain pose un problème car il peut se trouver en quantité importante (de 10 à 1000 mg par kg) dans certains aliments "appertisés" en boîtes de fer blanc par suite d'une corrosion lente de la boîte de conserve sous l'action de divers facteurs. De plus, il a été proposé que le chlorure stanneux soit considéré comme un additif nécessaire à la dose de 20 à 25 ppm; la Food and Drug Administration autorise d'ailleurs l'utilisation du chlorure stanneux, à la dose de 15 mg par kg.

Sur un plan fondamental, il a été indiqué que l'étain, absent chez les végétaux, se trouvait normalement dans certains organes humains, peut être en tant qu'oligo-élément essentiel au même titre que le nickel, le vanadium, le silicium ou le fluor. Sur le plan toxicologique, plusieurs expériences d'intoxication ont été réalisées chez différentes espèces animales qui présentent de sérieux troubles gastro-intestinaux.

A la vue de ces différentes données, et comme le tube digestif représente le premier organe-cible pour tout aliment, nous avons pensé qu'il était intéressant de vérifier tout d'abord si l'étain n'était pas capable de franchir les muqueuses digestives, puis de se répartir dans l'organisme, tout au moins à un faible pourcentage, car la majorité des auteurs soulignent le taux élevé de l'excrétion fécale de l'étain.

Notre travail a comporté les trois points suivants :

- 1 - Nous avons administré du chlorure stanneux en présence de différents composés nutritionnels susceptibles d'être associés à l'étain dans certains aliments.
- 2 - Nous avons incorporé du chlorure stanneux à la dose de 5000 ppm à l'alimentation de jeunes rats pendant un mois.
- 3 - Nous avons procédé, toujours chez le Rat, à une intoxication pendant six mois aux doses de 4000 et 8000 ppm.

Dans tous les cas, nous avons analysé la répartition tissulaire de l'étain, les principaux paramètres nutritionnels et hématologiques, ainsi que les images histologiques des organes essentiels.

Par les mesures de radioactivité effectuées, il apparaît comme certain que la barrière intestinale est pratiquement imperméable vis-à-vis de l'étain et cela dans les différentes conditions où nous nous sommes placés : soit en faisant varier les conditions d'administration, soit à court terme, soit à long terme. Nous rejoignons en cela les travaux et les conclusions de nombreux auteurs comme CALLOWAY et Mc MULLEN,

de GROOT et coll, HILES et, enfin, KUTZNER et BROD, pour ne citer que les principaux. Il est nécessaire de souligner, à ce propos, le rôle majeur de protection qu'exerce la barrière digestive, pour l'organisme, vis-à-vis de composés, tels que l'étain, qui peuvent se trouver dans l'alimentation.

L'étude nutritionnelle à laquelle nous avons procédé, lors des deux expériences d'intoxication, à court et à long terme, indique que l'étain induit un ralentissement de la croissance mais la diminution du gain de poids est en réalité une conséquence directe d'une diminution de consommation; les animaux en effet ont une inappétence pour le régime contenant de l'étain. De plus, comme l'étain n'est pas absorbé, la diminution de la croissance ne peut pas être due à un effet toxique organique mais plutôt à un effet sur la consommation ou sur la compétition avec l'absorption d'autres nutriments; à ce titre, les coefficients d'efficacité protéique, qui ne varient pas significativement, apportent la preuve que l'étain n'altère pas l'équilibre nutritionnel de l'organisme au niveau du métabolisme azoté. Il faut remarquer que ces différents effets s'observent aux trois doses étudiées (4000, 5000 et 8000 ppm), quelle que soit la durée du traitement, c'est-à-dire que le rat soit en croissance (intoxication d'un mois), ou après le traitement à long terme (six mois), c'est-à-dire à un moment où la croissance est théoriquement en palier. Par ailleurs, si on s'intéresse à l'évolution pondérale des quelques organes étudiés, on peut considérer que les modifications des poids du cerveau, des testicules, et du tissu adipeux péri-épididymaire ne sont que le reflet du retard de croissance provoqué par l'étain. Par contre, l'hypertrophie du coeur et celle de la rate, sans autre atteinte histologique, peuvent être la conséquence de l'anémie observée chez les animaux traités. Enfin, il faut surtout noter que le poids du foie, par rapport au poids corporel, reste remarquablement constant chez tous les animaux, ce qui signifierait que l'étain contenu dans l'alimentation ne déclenche pas une agression générale de l'organisme; on peut donc considérer que le tube digestif assure un rôle de barrière et de protection extrêmement efficace.

Lors d'études hématologiques, il a été noté que les animaux présentent une anémie marquée puisque le taux d'hémoglobine et l'hématocrite sont chaque fois diminués par rapport aux animaux témoins; il semble que l'étain pourrait agir de deux manières, d'une part en affectant la synthèse de l'hémoglobine, probablement par altération de l'absorption du fer et du cuivre, d'autre part en modifiant l'appétence ou la consommation alimentaire; des confirmations expérimentales seraient cependant nécessaires pour corroborer l'explication de tels mécanismes.

Enfin, les altérations histologiques observées dans le pancréas exocrine, déjà notées par DREEF VAN DER MEULEN et coll, peuvent s'expliquer par une certaine carence alimentaire car il est connu que les déficiences nutritionnelles induisent des modifications pancréatiques. Mais le tube digestif reste l'organe cible le plus touché par un régime contenant de l'étain: la muqueuse gastrique et la muqueuse intestinale s'enrichissent en mucus, signe d'une irritation et d'une réaction de défense de ces tissus; ces réactions sont à rapprocher des différents signes d'intolérance (nausées, brûlures d'estomac, vomissements, diarrhées etc ...) notés chez plusieurs espèces animales et chez l'Homme.

En conclusion, il ressort de cette étude que l'étain peut poser un problème sur le plan de la toxicologie alimentaire. Il apparaît comme certain que ce métal ne traverse pas la paroi intestinale, mais qu'administré à forte dose et de façon chronique, il entraîne une irritation générale des muqueuses gastro-intestinales et des phénomènes d'anémies globulaires. A l'heure actuelle, la dose journalière admissible du chlorure stanneux n'a pas encore été précisée (Rapport du Comité Mixte FAO/OMS d'Experts des Additifs Alimentaires, 1975) et le taux permissible de ce composé dans l'alimentation est d'environ 250 ppm. Cette valeur pourrait paraître raisonnable dans la mesure où les doses nécessaires pour mettre en évidence les manifestations toxiques précitées sont beaucoup plus élevées (4000 et 8000 ppm); d'autant plus que l'ingestion de l'étain par l'Homme se réalise uniquement lors de la consommation d'aliments appertisés dans des boîtes de fer blanc étamées. Mais, comme le souligne à très juste titre KNORR, on ne connaît pas suffisamment à l'heure actuelle les réactions chimiques de l'étain dans les aliments ainsi conservés; aussi serait-il souhaitable d'approfondir ce problème et de surveiller étroitement les conditions technologiques.

P. FRITSCH

G. de SAINT BLANQUAT

R. DERACHE

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Les résultats des travaux concernant ce présent contrat ont fait l'objet des publications suivantes :

- 1 - FRITSCH, P., de SAINT BLANQUAT, G., et DERACHE, R.
Effect of various dietary components on absorption and tissue distribution of orally administered inorganic tin in rats.
Fd. Cosmet. Toxicol., 1977, 15, 147-149.
- 2 - FRITSCH, P., de SAINT BLANQUAT, G. et DERACHE, R.
Etude nutritionnelle et toxicologique, chez le Rat, d'un contaminant alimentaire : l'étain.
Toxicology, 1977, sous presse.
- 3 - FRITSCH, P., de SAINT BLANQUAT, G. et DERACHE, R.
Impacts nutritionnels et toxicologiques de l'étain inorganique administré pendant 6 mois chez le Rat.
(à paraître).

Contractor : Istituto Superiore di Sanità

Contract n° : 043-74-1 ENV I

Project Leader : V.G. LONGO

Title of project : Electroencephalographic investigation of the effects of organophosphorous pesticides and their metabolites on the central nervous system

Neurological intoxication symptoms induced by large doses of anticholinesterase-type pesticides have been up to present time correlated with biochemical alterations, consisting of reduction in cholinesterase activity and consequent increase in brain acetylcholine levels. However, there is no clear evidence of correlation between any long lasting biochemical effects of single small doses of these drugs and behavioral impairment. In view of the importance of the cholinergic systems in the electrogenesis, recording of the brain electrical activity has been used as a sensitive parameter to show discrete alterations of the functional state of the brain induced by organophosphate pesticides. Electroencephalographic (EEG) changes more clearly demonstrate central than peripheral effects of drugs in animals; moreover, time series analysis of EEG using spectral techniques allows quantitative evaluation of spontaneous non-paroxysmal cerebral electrical activity.

Investigations were then initiated in our laboratory in an attempt to contribute to the development of threshold limit values for substances acting on the central nervous system.

Methods

Six rabbits were chronically implanted with cortical and hippocampal electrodes, and received 0.05, 0.10 or 0.15 mg/kg of methyl-parathion i.m. for at least 3 days. Sixty mice bearing chronically implanted cortical electrodes were challenged either with methyl-parathion (in doses of 0.125 to 4.0 mg/kg p.o. once a day for 3 days) or with physostigmine (in doses of 0.025 to 0.8 mg/kg i.p.).

The EEG tracings were recorded on paper and on magnetic tape, and after visual inspection were analyzed off-line by a digital computer. Power spectra of the EEG were computed, and were then divided in 6 frequency bands, ranging from 0.5 to 40 Hz. The mean and standard deviations of frequency bands in pre-treatment recordings for each subject were compared with those taken from similar time samples following drug or solvent, using statistical procedures (Fisher "F" test and Duncan test).

Results

Rabbits: Gross behavior and EEG tracings were normal on visual inspection in pre and post-drug recordings. Computer

analysis performed on the hippocampal tracing revealed an inconsistent increase in power of intermediate frequencies (7 - 12 Hz) followed by a decrease in power of intermediate and high frequencies.

Mice treated with smaller doses of physostigmine (0.05 and 0.1 mg/kg i.p.) or methyl-parathion (0.25 to 1.0 mg/kg p.o.) had normal exploratory behavior a few minutes following administration of drugs. On visual inspection desynchronized EEG tracings in both pre-drug and post-drug periods were shown. Larger doses of physostigmine (0.2 to 0.8 mg/kg) and methyl parathion (2.0 and 4.0 mg/kg) induced sluggishness, and EEG records appeared slower in frequency and lower in voltage. Computer analysis showed that small doses of physostigmine or methyl-parathion induced an enhancement in power of intermediate frequencies of the spectrum and of total power, whereas higher doses of both drugs induced a depression in power of several frequency bands and total power. Intermediate doses of physostigmine (0.1 - 0.2 mg/kg) induced a short-duration depression of the power in all bands of the spectrum, which was followed by an enhancement of low frequency bands, while intermediate doses of methyl-parathion induced an inconsistent increase of intermediate frequency band (7 - 12 Hz) followed by a long-lasting depression. All doses of physostigmine induced a transitory diminution of the mean frequency of the spectrum within 15 minutes following injection: this effect was significantly related to the dose.

Discussion

The lowest effective dose of methyl parathion, which can be detected through computerized EEG in mice, is as low as 0.25 mg/kg, i.e. about 0.5 - 1% of LD₅₀ (for methyl-parathion in mice is 39 mg/kg p.o.). The EEG effects of the "reversible" anticholinesterase drug physostigmine can be detected at higher doses (0.05 mg/kg i.p. corresponds to about 5 - 10% of LD₅₀ in mice). Studies are in progress to elucidate whether this and other discrepancies may be attribute to mechanisms different from cholinesterase inhibition in mammals brain.

This work has been realized with the contribution of the following people: E. Deodati, G. Gallozzi, A. Giuliani, C.J. Lindsey, A. Loizzo, E. Ortolani, S. Palazzesi, A. Pezzola, G.A. Zapponi.

References:

A. Loizzo, G.A. Zapponi, A. Giuliani, V.G. Longo
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A. Loizzo, C.J. Lindsey, G.A. Zapponi, V.G. Longo
Electroencephalographic analysis of subacute effects of methyl-parathion in the mouse. Comm. Int. Congr. EPIC IV, Hendersonville, N.C. U.S.A., 4 - 10 Apr. 1976

G. Gallozzi
Correlazione tra gli effetti comportamentali, biochimici, elettroencefalografici di pesticidi organofosforici nel topo. Doctoral thesis, Roma, Oct. 1977

Istituto Superiore di Sanità, Rome, Italy

Contract n. 069-74-1 ENV I

Project leaders: A. CARERE, G. MORPURGO and I. CAMONI

Mutagenicity of pesticides as pure compounds and after plant metabolism

The most important objectives of this project were the following:

- 1) to set up a battery of short-term tests able to reveal any kind of genetic damage induced by chemical agents. To this aim two prokaryotic microorganisms (Salmonella typhimurium and Streptomyces coelicolor) and one eukaryotic microorganism (Aspergillus nidulans) were chosen; the genetic events studied were point mutations, mitotic segregation (non-disjunction and crossing-over) and lethal recessives;
- 2) to calibrate the genetic systems with standard mutagens and to study the mutagenicity of pesticides as pure compounds; to this aim thirty five pesticides (Table 1) were tested; for some of them the relationship between chemical structure and genetic activity was also investigated;
- 3) to devise a system based on "in vitro" Nicotiana cell cultures which allows to study the possible effects of plant metabolism on the genetic activity of pesticides.

The back-mutation genetic assay developed by AMES et al. (PNAS 70, 782-786, 1973) in S.typhimurium was employed with strains TA1535, TA1536, TA1537, TA1538 and, only recently, TA98 and TA100 in the presence and in the absence of rat liver microsomal fractions.

The genetic system set up in S.coelicolor is a forward-mutation system based on the induction of resistance to low levels of Streptomycin; this system indicates recessive mutations belonging to at least three cistrons. The test was calibrated with direct or indirect alkylating agents to which it was particularly sensitive. In addition, the system was able to detect frame-shift mutagens (2-AF and 2-AAF). The test may be successfully applied also in the presence of rat liver microsomal fractions in the same manner as in S.typhimurium.

For the study of point mutations in A.nidulans two different forward-mutation systems were used: 8-Azaguanine resistance and induction of suppressor mutations of the met-1 marker. These genetic systems are clearly sensitive to standard mutagens known to induce mutations of the base-substitution type; it is not yet known if they are able to detect frame-shift mutations. The induction of mitotic segregation was studied by using a selective method previously described by BIGNAMI et al. (Mutation Res. 26, 159-179, 1974). Mitotic non-disjunction was studied also with a non selective method described by FRATELLO et al. (Genetics 45, n.6, 1960). Finally, a method to test the appearance of lethal recessives in A.nidulans was set up (G.MORPURGO et al., Canadian J.Genetics, in press).

The mutagenicity experiments were performed in the three microorganisms mainly using a "Spot test" technique applied with triangles of absorbent paper (BIGNAMI et al., 1974). In some cases "Plate test" and "Liquid test" were also used.

In order to study the effects of plant metabolism on the genetic activity of pesticides a method, based on the use of Nicotiana cells cultivated in liquid culture (Skoog's medium) was set up. Homogenates of Nicotiana cells grown in the presence of a given pesticide can be used either for mutagenicity studies with "in vitro" microbial genetic systems or for chemical analysis. With the purpose of verifying if the metabolism of "in vitro" Nicotiana cell cultures simulates in a good manner the plant metabolism occurring in the field chemical studies with five pesticides (Atrazine, Dichlorvos, Kelevan, Gardona and Maleic Hydrazide) were performed.

Results

The results obtained after mutational studies have shown that among the pesticides investigated (Table 1) seventeen turned out to be mutagenic in at least one genetic system (Table 2).

Among the pesticides tested, some were studied

more carefully in order to try to correlate their mutagenic activity with their chemical structure. These pesticides belonged to two different chemical classes (phosphoric esters and carbamates) and contained in their molecule a chlorinated vinyl or allyl group. Dichlorvos, which is a phosphoric ester with a dichlorovinyl (vinylidene chloride) group as side chain, and Trichlorfon, known for its spontaneous conversion in Dichlorvos, were both mutagenic in Salmonella, Streptomyces and Aspergillus; five organophosphorus pesticides (Azinphosmethyl, Fenchlorphos, Mevinphos, Monocrotophos and Parathion methyl), similar to Dichlorvos but devoid of the chlorinated group, were not mutagenic. Similarly, three carbamates -Diallate, Triallate and Sulfallate- which contain in their molecule a chloroallyl group were mutagenic while two other carbamates (EPTC and Noruron) devoid of the allyl group were not mutagenic. These results strongly suggest that the presence of a vinylidene chloride or allyl chloride group in the molecule of the pesticides investigated is responsible for their genetic activity.

By means of gas-chromatographic and chromatographic techniques it was demonstrated that four pesticides -Atrazine, Dichlorvos, Gardona and Kelevan-were rapidly metabolized by Nicotiana cells, while Maleic Hydrazide was not metabolized. These results suggest that "in vitro" Nicotiana cell cultures accurately simulate the plant metabolism occurring in the field.

The results obtained allow the following considerations:

- 1) some aspects remain to be clarified (e.g. the problem of the frame-shift mutations in Aspergillus); however, all the genetic systems are well set up and can be routinely used;
- 2) the S.coelicolor forward-mutation test seems to be well suited for environmental chemical mutation studies;
- 3) the use of A.nidulans for the induction of genetic events as mitotic crossing-over and non-disjunction and also for the recent possibility to study the induction of lethal recessives, is particularly interesting;

- 4) the results obtained add further information about the genetic activity of many pesticides previously tested by other authors and new evidence on pesticides which had not yet been investigated;
- 5) for some of the pesticides tested the relationship between chemical structure and genetic activity was also investigated;
- 6) with the exception of a few cases (Dichlorvos and Trichlorfon in Salmonella; Captan in Streptomyces) the mutational response obtained with the "Spot test" technique was always clearcut in comparison with other techniques; in some cases (e.g. with fumigants or chemicals acting through vapours) the response obtained with the "Spot test" was clearer than that obtained with "Plate test" procedures;
- 7) the results obtained with Atrazine, Dichlorvos, Gardona, Kelevan and Maleic Hydrazide suggest that the Nicotiana cell cultures closely simulate the metabolism of the whole plant in the field. The method is easy, rapid, unexpensive and could be applied to "in vitro" microbial genetic systems.

Preliminary accounts of the results obtained were presented at the last three EEMS meetings (Firenze 1975, Gernrode 1976, Edinburgh 1977) and at the last three Italian meetings (Associazione Genetica Italiana, Parma 1975, Pisa 1976 and Napoli 1977). Moreover the results obtained were the object of the following manuscripts:

- 1) A.Carere, V.A.Ortali, G.Cardamone and G.Morpurgo. Mutagenicity of Dichlorvos and other structurally related pesticides in Salmonella and Streptomyces. Submitted to Chemico-Biological Interactions.
- 2) A.Carere, V.A.Ortali, G.Cardamone, A.M.Torracca and R. Raschetti. In vitro microbiological mutagenicity studies of pesticides. Submitted to Mutation Res.
- 3) M.Bignami, F.Aulicino, A.Velcich, A.Carere and G.Morpurgo. Mutagenic and recombinogenic action of pesticides in Aspergillus nidulans. Mutation Res. in press.
- 4) G.Morpurgo, S.Puppo, L.Conti and G.Gualandi. A quick method for testing recessive lethals with a diploid strain of A.nidulans. Submitted to Canadian J.Genetics.
- 5) M/Bignami, G.Cardamone, A.Carere, P.Comba, E.Dogliotti, G.Morpurgo and V.A.Ortali. Mutagenicity of chemicals of industrial and agricultural relevance in Salmonella, Streptomyces and Aspergillus. Submitted to Cancer Res.
- 6) G.Morpurgo, F.Aulicino, M.Bignami, L/Conti and A.Velcich. Relationship between the structure and the mutagenicity of Dichlorvos and other pesticides in Aspergillus nidulans. Accademia Nazionale dei Lincei, in press.

TABLE 1. CHEMICALS INVESTIGATED (COMMON NAME)

ACROLEIN	ETHYLENE DICHLORIDE
ALLYL ALCOHOL	ETHYLENE GLYCOL
AMINOTRIAZOLE	FENCHLORPHOS
AZINPHOSMETHYL	IOXYNIL
BARBAN	MECOPROP
BENOMYL	MEVINPHOS
CAPTAFOF	MONOCROTOPHOS
CAPTAN	NEBURON
CARBON TETRACHLORIDE	NORURON
DALAPON-Na	PARAQUAT
DIALATE	PARATHION METHYL
DICHLORVOS	PICLORAM
DINOBTION	PROPYLENE DICHLORIDE
DIQUAT	SULFALLATE
DODINE	TRIALATE
EPTC	TRICHLOROACETIC ACID
ETHYLENE DIBROMIDE	TRICHLORFON

TABLE 2. CHEMICALS WITH MUTAGENIC ACTIVITY IN SALMONELLA, STREPTOMYCES, ASPERGILLUS

Compound	<u>S.typhimurium</u>	<u>S.coelicolor</u>	<u>A.nidulans</u>		
			point mutat.	c.o.	n-d
Acrolein	+ (TA1538)	+	-	-	-
Aminotriazole	-	+	-	+	+
Benomyl	-	-	-	-	+
Captafol	-	-	+	+	-
Captan	+ (TA1535)	+	+	+	-
Diallate	-	+	+	+	-
Dichlorvos	+	+	+	+	+
Diquat	- (TA1535, TA1538)	nt	nt	nt	nt
Ethylene dibr.	+ (TA1535)	+	+	-	-
Ethylene dichl.	+ (TA1535)	-	-	-	-
Paraquat	- (TA1535, TA1538)	+	nt	nt	nt
Picloram	-	+	-	-	-
Propylene dichl.	+ (TA1535)	-	+	-	-
Sulfallate	+ (TA1535)	+	+	+	-
Tordon	-	+	-	-	-
Triallate	+ (TA1535)	+	+	+	-
Trichlorfon	+ (TA1535)	+	+	-	-

This qualitative response was obtained with a "Spot test" technique (see Test) for all compounds, with the following exceptions: Dichlorvos and Trichlorfon in Salmonella (Liquid test); Captan in Streptomyces (Plate test).

nt = not tested; c.o. = crossing over; n-d = non-division

Contractor : University of Leiden

Contract no.: 030 - 74 - 1 ENV NL

Project leader: Prof.Dr. F.H. Sobels

Title of project: Studies on the induction of genetical damage by
chemical mutagens in Drosophila.

Two of the most important objectives of environmental mutagen testing with Drosophila concern (i) the question of whether Drosophila is capable of identifying suspect chemicals that might pose a genetic threat to man, and (ii) whether this system will be a reliable and realistic model for assessing their effects. To achieve these objectives attention has been focused on (i) examining the extent of correlation between different measured end points of genetic damage, for given mutagens at different concentrations ranging from the Lowest Effective Concentration (LEC) to LD₅₀ and (ii) testing of suspected mutagens (carcinogens) which belong predominantly to the indirectly acting type, that is, those which require enzymatic bioactivation before they become mutagenic.

The present status of our work leads us to conclude that recessive lethal assays provide the most reliable and sensitive tool for mutagen testing in Drosophila. A wide array of suspect mutagens can be identified when using the induction of recessive lethal mutations as measure of mutagenicity. The situation becomes far more complicated when chromosome breakage by chemical mutagens is considered (Table 1). A still growing number of mutagens elicit clear-cut activity when assayed for point mutation induction, but escape detection in tests on chromosome breakage. The precarcinogen diethylnitrosamine (DEN) may be quoted as an example. Other mutagens (carcinogens) induce chromosome breakage at subtoxic and toxic concentration only, whereas recessive lethal mutations arise far below LD₅₀ levels (Table 1). The major conclusion from these comparative studies has been that there are Two Effective Concentration Levels, that is, lower concentrations are required for the induction of recessive

lethal mutations (predominantly gene mutations), than for producing chromosome breakage events. Recessive lethal assays thus should form the core of any testing programme with *Drosophila*.

Drosophila has the enzymes needed for the transformation of precarcinogens into reactive metabolites. The mutagenic effectiveness of various types of precarcinogens in recessive lethal tests provides strong support for the considerable activating potency of *Drosophila*.

Future work aims at the development of mutagen-sensitive strains and a comparison in a model population of the induction and accumulation of genetic damage after short-term vs. long-term exposure.

Completed studies funded by E.C. contract No. 030 - 74 - 1 ENV NL

No.

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2. Blijleven, W.G.H., and E. Vogel, The mutational spectrum of procarbazine in *Drosophila melanogaster*, *Mutation Res.*, 45 (1977) 47-59.
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7. Sobels, F.H., and E. Vogel, The capacity of *Drosophila* for detecting relevant genetic damage, *Mutation Res.*, 41 (1976) 95-106.

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9. Sobels, F.H., Some problems associated with the testing for environmental mutagens and a perspective for studies in "Comparative Mutagenesis", *Mutation Res.*, 46 (1976) 245-260.
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Table 1. Mutagenic activity in *Drosophila* sperm as a function of the endpoint of genetic damage used as indicator

Compound	Concentration range tested (mM)	LD ₅₀ (mM)	<u>Recessive lethals</u>		<u>Dominant lethals</u>		<u>Chromosome loss^{a)}</u>		<u>Translocations</u>	
			Act.	LEC:LD ₅₀	Act.	LEC:LD ₅₀	Act.	LEC:LD ₅₀	Act.	LEC:LD ₅₀
MMS	10 ⁻² - 2.0	~5.0	+	1 : 100	+	1 :	+	1 : 10	+	1 : 10
TEB	10 ⁻⁶ - 10 ⁻²	> 0.1	+	<1 : 6700	+	1 : 1000	+	1 : 200	+	1 : 1000
2,4,6-Cl ₃ -PDMT	10 ⁻³ - 2.0	~ 2.5	+	1 : 1000	(+)	1 :	(+)	1 : 2	(+)	1 : 4
DEN	10 ⁻² - 10.0	~10.0	+	1 : 100	0 !		0 !		(+)?	at LD ₉₀
Procarbazine	0.5 - 30.0	> 30.0	+	<1 : 60	(+)?	1 :	(+)?	1 : 1	0	
Cyclophosphamide	1.0 - 20.0	> 20.0	+	<1 : 20	0		0			not tested
Trofosfamide	1.0 - 20.0	> 20.0	+	<1 : 20	0		0			not tested
Ifosfamide	1.0 - 20.0	> 20.0	+	<1 : 20	0		0			not tested
Vinyl chloride	13.6 - 800.0	>800.0	+	<1 : 60	0		0		0,(+)?	1 : 2

Abbreviations: Act., mutagenic activity; 0, non-mutagenic; +, mutagenic; (+) mutagenic at toxic concentrations; (+)?, activity questionable; LEC, Lowest Effective Concentration; MMS, methylmethanesulfonate; TEB, 2,3,5,6-tetra-ethyleneimino-1,4-benzoquinone; DEN, diethylnitrosamine.

a) Loss of X- or Y-chromosome.

Contractor : University of Leiden

Contract No.: 030 - 74 - 1 ENV N

Project Leader: Prof.Dr. F.H. Sobels

Title of project: Studies on the induction of point mutations by chemical mutagens in mammalian cells.

Introduction

The aim of the research with mammalian cells in vitro was to evaluate the mutagenic potential of mutagens using point mutations in L5178Y mouse lymphoma cells, V-79 Chinese hamster cells and human diploid skin fibroblasts as genetic endpoint. This involved a further development of these test systems, the testing of some known and some potential mutagens and the development of parameters for the comparison of mutagens, markers and test systems.

Development of methods

A mutational assay system was developed for L5178Y mouse lymphoma cells using HGPRT (hypoxanthine-guanine-phosphoribosyl-transferase)-deficiency and TK (thymidine kinase)-deficiency as markers. Reconstruction experiments, in which mutant cells were mixed with wild type cells, indicated which cell density had to be used during selection. Expression time experiments provided information on the time period needed for complete expression of induced mutants. The mutational assay system using human diploid skin fibroblasts was improved by the application of correction factors for variations in the experimental conditions. Activation of indirect mutagens was achieved by the use of rat liver homogenates.

Screening of known and potential mutagens

The following known mutagens which act directly, that is without metabolic activation, were tested and found to be mutagenic: EMS, MMS, MMC and DEB. The dose-response relationships were all linear. A linear dose-response relationship was also found for the potential mutagen epichlorohydrin applied without metabolic activation. The drugs DMN, DEN and

Natulan proved to be mutagenic only after metabolic activation. The dose-response relationships were non-linear: for DMN and DEN a curve was found which reaches a plateau and for Natulan a humped curve was found. An explanation for these curves could be sought in saturation of the activation system for DMN and DEN and in the variety of drug metabolizing pathways which are open for Natulan. Preliminary data indicate that MMC is inactivated by the rat liver homogenate. Comparison of the induced mutant frequencies for TK-deficiency and HGPRT-deficiency, determined simultaneously in the same L5178Y cell line showed that the induction at the TK-locus is 1.7 - 3.3 times higher than at the HGPRT-locus. This ratio depends on the mutagenic agent used. The mutation induction at the HGPRT-locus of L5178Y mouse cells and V-79 Chinese hamster cells appears to be about the same. The L5178Y cells were more sensitive than the V-79 cells for the toxic action of the drugs.

Comparison of mutagenic potential

As data have been collected for different test systems and different markers the problem arises of how to compare the mutagenic potential of the mutagens. One way of comparing the mutagenicity of compounds is the calculation of the mutation induction per mM per hour of treatment (Table 1). Because the actual treatment time in the experiments was two hours and because a concentration of 1 mM was not always used this parameter is an extrapolation which could be inaccurate. However the advantage of this parameter is that it will facilitate the comparison of data from authors which use different treatment conditions. For the calculation of this parameter the shape of the dose-response curve has to be taken into account. For directly acting compounds which are characterized by a linear dose-response relationship a rather reliable estimate of the mutagenic potency is obtained if the compound is stable during the treatment period. For indirectly acting compounds the concentration of the active form is not known, therefore the concentration of the inactive precursor has been taken for the calculation of the mutagenic potency. For the curves which show a saturation of the mutagenic effect (DMN, DEN) the initial slope of the curve was used for the calculation of the parameter. For the humped dose-response curve (Natulan^R) the maximum induced mutant frequency was

used for the calculation. The mutagenic potencies (MP) obtained in this way are given in Table 1. This table shows that the bifunctional agents (MMC and DEB) are characterized by much higher MP's than the monofunctional agents. Comparison of EMS and MMS shows the same MP, although in experiments with EMS many more mutants can be obtained than with MMS. The explanation for this is the much higher toxicity of MMS. To take this information into account another parameter has to be used which relates mutation induction to cell killing. Therefore in Table 2 the mutation induction at LD₅₀ has been given.

Abbreviations:

EMS, Ethylmethanesulfonate
 MMS, Methylmethanesulfonate
 MMC, Mitomycin C
 DEB, Diepoxybutane
 DEN, Diethylnitrosamine
 DMN, Dimethylnitrosamine

Completed studies funded by E.C. Contract No. 030 - 74 - 1 ENV N

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Table 1. Mutagenic potencies of the chemical mutagens x 10⁵

Cell line:	L5178Y	L5178Y	V-79
Locus :	TK	HGPRT	HGPRT
Compound			
EMS	5.0	2.3	0.9
MMS	-	1.1	2.2
DEB	185	55	-
MMC	-	-	2600
epichlorohydrine	-	2.7	-
DMN*	-	1.4	-
DEN*	-	2.8	0.6
Natulan*	2.6	1.2	2.3

* after metabolic activation.

- not tested

The mutagenic potency of a compound is expressed as induced mutant frequency per mM per hour of treatment.

Table 2. Mutation induction at LD₅₀ x 10⁵

Cell line:	L5178Y	L5178Y	V-79
Locus :	TK	HGPRT	HGPRT
Compound			
EMS	36.0	15.8	89.6
MMS	-	0.5	6.6
DEB	4.0	1.2	-
MMC	-	-	6.3
epichlorohydrine	-	1.9	-
DMN*	-	4.2	-
DEN*	no toxicity observed		
Natulan*	no toxicity observed		

* after metabolic activation

- not tested

Contractor: University of Leiden; Contract No.: 030 - 74 - 1 ENV N; Project leader: Prof.Dr. F.H. Sobels
 Summary table on mutagenicity of MMS, DEN, and Natulan^R (procarbazine) in various assay systems

COMPOUND	DROSOPHILA in vivo					MAMMALIAN CELLS							
						Mice in vivo			Mouse lymph. in vitro		Chinese hamster in vitro		
	point mutat.	DL	TR	TCL	PCL	BM Chr.ab.	BM Micron.	SPG Chr.ab.	point mutations HGPRT ⁻	TK ⁻	chromatid aberr.	SCE's	point mutations HGPRT ⁻
MMS	+	+	+	+	+	?	+	?	+	n.t.	+	+	+
DEN	+	0	0 ^{b)}	0	0	0	0	n.t.	+ ^{a)}	+ ^{a)}	+ ^{a)}	+ ^{a)}	+ ^{a)}
Natulan	+	(+)	(+)	+	?	+	+	+	+ ^{a)}	+ ^{a)}	0 ^{a)}	+ ^{a)}	+ ^{a)}

a) after metabolic activation

b) 1 translocation at LD₉₀ (1 in 670 gametes)

BM, bone marrow; SPG, spermatogonia; Chr.ab., chromosome aberrations; SCE, sister-chromatid exchange; n.t., not tested; DL, dominant lethals; TR, 2;3-translocations; TCL, total chromosome loss (X or Y); PCL, partial Y-chromosome loss.

Contractor : University of Leiden

Contract No.: 030 - 74 - 1 ENV N

Project leader: Prof.Dr. F.H. Sobels

Title of project: Studies on the induction of chromosome aberrations in mammalian germ cells and somatic cells by chemical mutagens.

Introduction

Studies with lower organisms and *Drosophila* are important for detecting the mutagenic effect of environmental chemicals, but obviously it is essential to explore to what extent these findings are applicable to mammals, in particular man. Therefore, a variety of mammalian cytogenetic assay systems was used to study the effect of chemicals with known or suspected mutagenic action in lower organisms. With these systems we have performed, in the past three years, experiments (1) to establish whether information on induced cytogenetic damage in somatic cells has predictive value for similar damage in germ cells, (2) to evaluate the relative merits of the existing cytogenetic parameters to evaluate mutagenicity, and (3) to develop an in vitro system for the detection of mutagens requiring bioactivation so as to allow a better comparison between in vivo and in vitro data.

In vivo experiments

A study was made of cytogenetic damage induced in male mice by a wide range of concentrations of CNU-ethanol (2-chloroethyl-1-nitrosoureido-ethanol), BCNU (2-chloroethyl)-3-nitrosourea), methylmethanesulfonate (MMS), diethylnitrosamine (DEN), mitomycin C (MMC) and procarbazine (Natulan^R). Induced damage was evaluated by determining frequencies of (1) micronuclei in polychromatic erythrocytes of bone-marrow, (2) chromosome aberrations in bone-marrow, (3) chromosome aberrations in spermatogonia, and (4) reciprocal translocations induced in spermatogonia and scored in spermatocytes (for BCNU and CNU-ethanol only).

The first three parameters were always studied from the same animal

and correlation coefficients were calculated at each concentration of the tested chemical. Positive correlations could not be detected. However, the induction of damage at a particular concentration has been studied only at one or two sampling times after treatment. Thus, it remains possible that selection of other time intervals for a given concentration would result in some positive correlations. Furthermore the presence of a positive correlation at one concentration does not necessarily mean that such a correlation does exist at other concentrations, because concentration dependent mitotic delay and selective cell killing might differentially affect the concentration-effect curves for the parameters under study.

On the basis of the present data we conclude that information obtained by the routine application of one cytogenetic test cannot be used to make quantitative predictions about other types of damage and/or the same type of damage in different types of cell in the same animal.

We also explored which routine cytogenetic test would be the most sensitive one for demonstrating the clastogenic effect of chemicals. For this purpose these tests were performed in the way they are routinely used (Ref. 4). Under these conditions the order of sensitivity for the study with BCNU and CNU-ethanol was: micronuclei > chromosome aberrations in bone-marrow > chromosome aberrations in spermatogonia > translocations in spermatocytes. The picture for procarbazine is roughly similar, but for the other chemicals tested it is not yet well established. DEN did not induce any type of cytogenetic damage. MMS was only tested with the bone-marrow tests and of these the micronucleus test was superior. MMC was only tested at one concentration.

In vitro experiments

Since most mutagens are clastogenic in higher organisms and because most of the mutagens are carcinogens, cytological test systems are potentially useful for the pre-screening of mutagens and carcinogens in mammals. This is particularly true for the relatively quick in vitro systems. Until recently, in vitro systems suffered from the drawback that indirectly acting mutagens could not be detected because a microsomal-mediated cytogenetic assay was not available. Recently, we have developed a liver microsomal system in vitro that permits the activation and subsequent

detection in Chinese hamster ovary cells of indirectly acting mutagens (Table 1). In the presence of the rat liver microsomal system dimethylnitrosamine and diethylnitrosamine were extremely potent in inducing chromosome aberrations and sister chromatid exchanges (SCE's), whereas chromosome aberrations could not be detected with DEN *in vivo*. Procarbazine also increased the frequency of SCE's in the presence of the microsome system but the situation for chromosome aberrations is not very clear. Methylphenylnitrosamine (MPNA) slightly increased the frequency of SCE's in the presence of microsomes but chromosome aberrations could not be detected. Preliminary data seem to indicate that this compound increases the frequency of micronuclei when administered *in vivo*. An *in vitro* experiment with atrazine was fully negative. MMS and EMS which do not require bioactivation were also tested. Both compounds increased the frequencies of SCE's and in line with expectation the frequencies were the same in the presence and absence of rat liver microsomes.

Completed studies funded by E.C. Contract No. 030 - 74 - 1 ENV N

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Table 1. Summary table on chromosome aberrations and sister chromatid exchanges (SCE's) induced by chemical mutagens in Chinese hamster ovary cells in the presence and absence of rat liver microsomes

Compound	Concentration range (mM)	Chromosome aberrations	SCE's / cell (max. freq.)		
			control	induced	
				-micr.	+micr.
MMS	0.05 - 0.50	+	11	63	65
EMS	0.04 - 15	-	10	24	24
DEN	4 - 200	+	11	10	30
DMN	3 - 135	+	11	10	63
MPNA	1.3 - 10	-	10	9	12
Atrazine	12.5 - 100	-	9	8	9
Procarbazine	0.05 - 10	?	11	10	27

Contractor : Etat belge (Ministère de la Santé Publique), Institut d'Hygiène et d'Epidémiologie, représenté par M. D'HOORE, Ministère de la Santé Publique.

Nr of Contract : CEE - O40 - 74 - 1 ENV. B.

Chief of Project : Dr. R.F. MOUTON

Title of Project : DNA MISREPAIR AND CARCINOGENESIS : Latent Cytotoxicity of Repair Inhibitors of the Human Environment.

General Description of the work performed :

1. AIM.

A first contribution to the assessment of the global (1) genetic toxicity risk linked to the exposure of Man to chemicals suspected to act synergistically, this with hope to develop quick prescreening "synertest" of carcinogenicity plus co-carcinogenicity.

Synergy can be anticipated and predicted on the basis of fundamental knowledge regarding the mechanisms (2) which determine the fate of pre-mutational lesions of DNA. In particular, the binary synergy between DNA insulting chemicals capable to induce repair (3,4) and DNA repair inhibitors capable to block repair and transform error-free repair into error-prone repair i.e. mutation including carcinogenic somatic mutation when the insulting agent is a well known carcinogen (5,6).

We have used well known radiation lesion repair inhibitors with the hope that these drugs would act also on DNA repair induced by chemicals instead of radiations. These chemicals are either those used in chemotherapy (MEL; NAT; MMC) or those given as European reference carcinogens ° to the 15 CEC coordinated laboratories aiming to develop suitable battery of quick prescreening tests for potentially dangerous chemicals present in association in the Environment, Man appearing as an integrator of nuisance.

2. MATERIAL and METHODS.

2.1 Biological systems used for our synertest :

Sub-mammalian : the eucaryotic, monocellular protozoan ciliate *Tetrahymena pyriformis* GL presents many specific advantages : high repair capacity, capability to absorb insoluble chemicals through their cytostome, high content in DNA, probably having their own activating system.

Mammalian : while we were building our mammalian/human cell culture unit, we have confined our study to a human monolayer cell culture (mouth epithelioma KB in continuous line) utilised since 15 years by our host Virology Section. Preliminary results designed to check the subtoxicity of Caffeine has led not only to our expected blank, but, in specific conditions, to a dramatic stabilising effect of our culture which interests virologists aiming to detect slow viruses and cancerologists aiming to obtain a stabilization of cancerous cells. We tentatively try to explain these results by an inhibition of phosphodiesterase with consequent raise of c-AMP level (7).

° MMS, DEN, NAT, MMC, ATR.

2.2 Adapted or developed methods for our synertest :

2.2.1 Growth curves : the method used so far for testing the repair inhibiting capacity of chemicals after UV irradiation of *Tetrahymena pyriformis* has been adapted to DNA insulating chemicals (for experimental details, see the progress reports). The replacement of the UENO's growth medium by ZEUTEN's medium resulted in an increase of the growth rate (2H30 instead of 5H) but at the cost of one generation (5 instead of 6).

2.2.2 Survival curves : the development of a new method for cloning *Tetrahymena* in microdrops within agar-gelatin semi-solid medium in Petri dishes allowed us to obtain true survival curves (8,9,10).

2.2.3 U.V. photolesions detection : the development of an immunocytological method for detecting directly DNA UV photolesions, in situ, has been extended to *Tetrahymena* from mammalian cells (11).

3. RESULTS.

3.1 Prescreening synertests : *T. pyr.* growth curves. TABLE 1a.

		DNA INSULTING AGENTS						
		T	UV 900 ergs/2 mm	MMC [°] 1,2x10 ⁻³	MEL ⁻³ 5x10 ⁻³	MMS ⁻² 2x10 ⁻²	DEN ^{°°} 1,4x10 ⁻⁴	NAT ^{°°°} 1x10 ⁻²
D N A R E P A I R	T	-	±	±	±	±	±	±
	CAF ⁻³ 1x10 ⁻³	+	+++	+++	-	+++	+++	+
	CAF ⁻⁴ 2x10 ⁻⁴	-	++	++	+	±	±	+
	Q 1,2x10 ⁻⁵	-	++	+++	++	+	++	++
	C1Q 5x10 ⁻⁵	-	++	±	±	-	-	-

° : see fig. 1a

°° : used without Ames'mix. Weak mutagen, according to Ames

°°° : id. no mutagenic, according to Ames

- : no effect at 48H, 28° C

± : slight synergistic effect at 48H, 28° C

+ : positive synergistic effect at 48H, 28° C

++ : marked synergistic effect at 48H, 28° C

+++ : strong synergistic effect at 48H, 28° C

3.2 Prescreening synertests : T. pyr. survival curves. TABLE 1b.

D N A H I B I T O R S		DNA INSULTING AGENTS			
		T	UV 450 ergs/ mm ²	MMC ^o 1,2x10 ⁻³ M	MMS ⁻² 2,6x10 ⁻² M
	T	100%	80%	30%	46%
	CAF 1x10 ⁻³ M	100%	24%	18%	26%
	Q 1,2x10 ⁻⁵ M	100%		22%	45%

^o : see fig. 1b

% : percentage of survival clones, after 3 days.

3.3. Prescreening synertests : UV induced lesions with radiolabelled antibodies.

The sensitivity and specificity of the technique appear to be excellent on autoradiography of the tritiated DNA bound antibodies. For our synertest, we may thus correlate directly the amount of non-repaired lesions and the repair inhibitor effect. See fig. 2 for UV + CAF.

Application to MMS lesions to which anti-UV photolesions antibodies were ineffective, is already under investigation.

4. CONCLUSIONS.4.1 Sub-mammalian synertest :

Two of the three UV reference anti-repair agents so far tested at sub-toxic levels have shown a synergistic effect. Further assays are needed to precise the effect of chloroquine. These results are supported by two independent biological methods and as far as UV + CAF is concerned are further confirmed by a third direct radioimmunoassay.

4.2 Mammalian synertest :

Preliminary results obtained in collaboration with our Virology Section have revealed the complexity of the effect of sub-toxic concentrations of caffeine on a human cells culture.

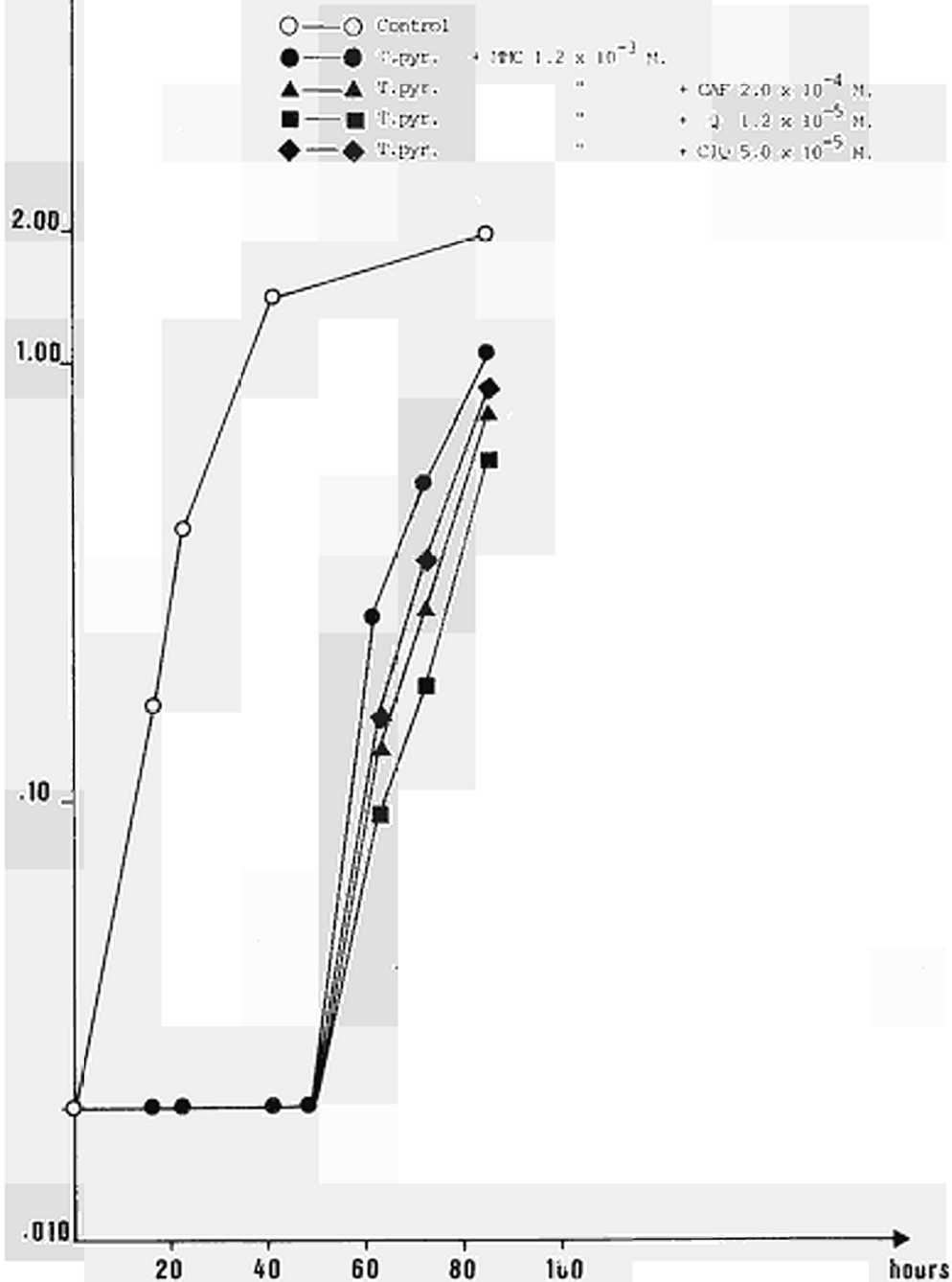
Monolayer survival obtained unexpectedly in our control experiment has already been applied to the detection of slow human viruses with an 100 fold increase of detection sensitivity (12).

5. LIST OF PUBLICATIONS.

- Tetrahymena news 1974, 2 (6) : 1
- Journal of Protozoology 1975, 22 (3) : 84A
- International Journal of Radiation Biology 1976, 29 (6) : 573
- EORTC News Letters 1976 Special Issue n° 48
- Archives of Virology 1976, 52 : 263-268
- II Congresso Nacional, Academia Mexicana de Ciencia y Tecnologia Nucleares A.C. 1975 (film)
- Journal of Protozoology 1977 (submitted).

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Fig. 1a : GROWTH CURVES OF *T. pyriformis*.

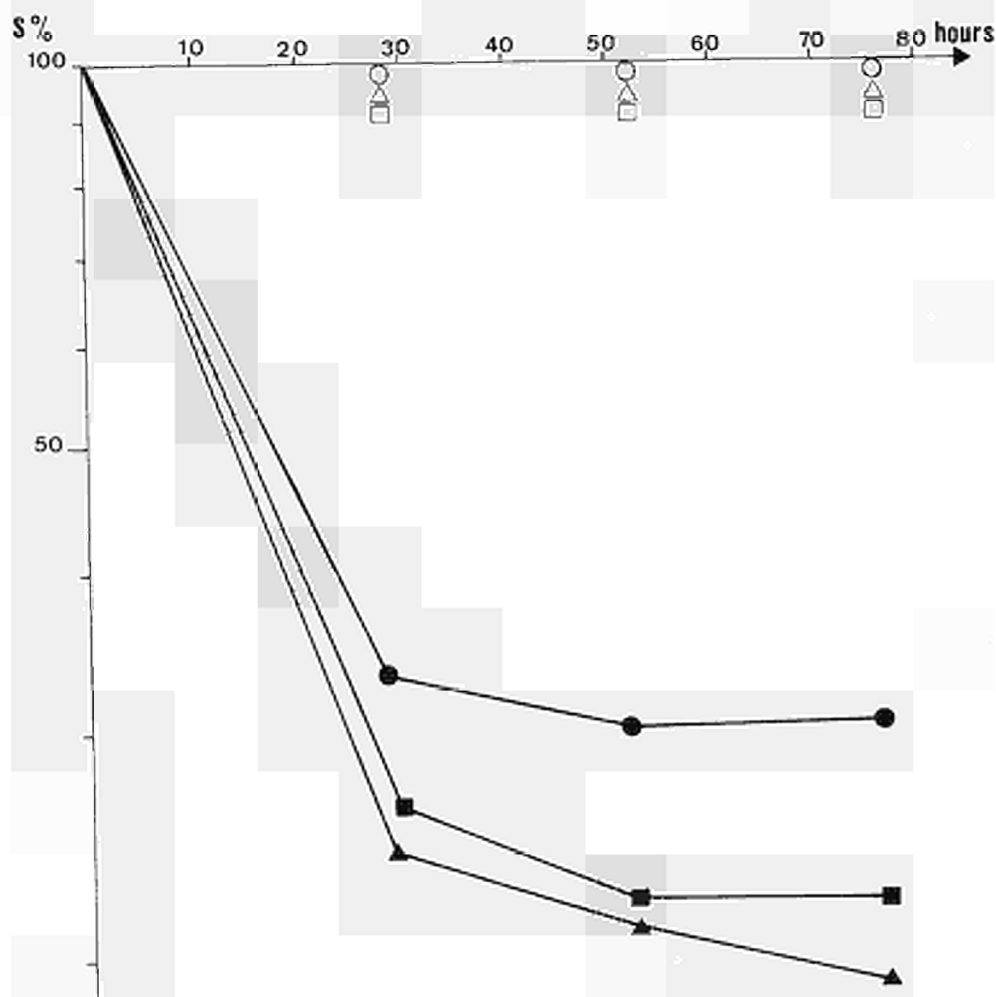
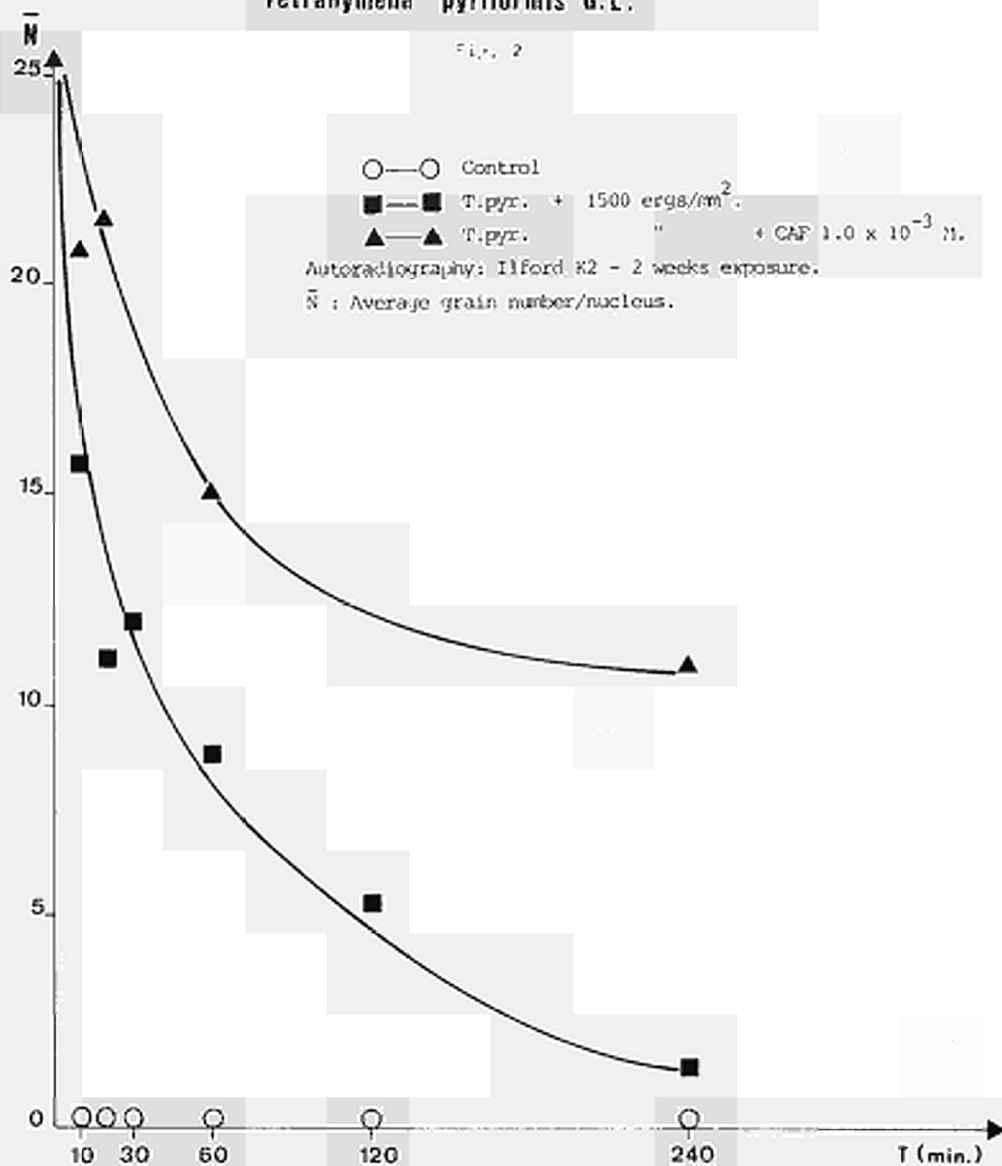


Fig. 1b : SURVIVAL CURVES OF *T. pyriformis*.

- Control
- △—△ Control + CAF 1.0×10^{-3} M.
- Control + Q 1.2×10^{-5} M.
- *T. pyr.* + MMC 1.2×10^{-3} M.
- ▲—▲ *T. pyr.* + " + CAF 1.0×10^{-3} M.
- *T. pyr.* + " + Q 1.2×10^{-5} M.

PHOTOPRODUCTS REPAIR IN THE DNA OF UV-IRRADIATED
Tetrahymena pyriformis G.L.

Fig. 2



Contractor University of Aston in Birmingham

Contract N^o 029-74-1 ENV UK

Project Leader : Professor D.G. Wibberley

Title of Project : Investigation into the Correlation between
the Quantities of Substances of Possible Toxic
Hazard present in Placenta and Congenital
Malformations.

Satisfactory analytical procedures have been developed for the determination of lead, cadmium, phthalate esters and selenium in placental samples and a tissue solubilisation procedure with Soluene-350 established.

Attempts have been made to check whether there is any correlation between the levels of these environmental hazards and congenital malformations.

Tissue Solubilisation by Soluene - 350 (T.M.)

Sample preparation for lead determinations by graphite furnace atomic absorption spectrophotometry.

The use of Soluene - 350 was investigated for the solubilisation of human placental tissue in order to determine the lead content by graphite furnace atomic absorption spectrophotometry. The advantages of this method over classical methods of sample preparation were that the detection limit for this method is 0.025 ppm and relative standard deviations for placental tissue was 7.3% and that the method combines the inherent specificity and simplicity of atomic absorption analysis with the greatly increased sensitivity of the heated graphite tube atomiser to provide a rapid and simple method for lead determinations in animal tissue.

Determination of Di-(2-Ethylhexyl)phthalate in Human Placenta

A simple method has been devised for the analysis of di-(2-ethylhexyl) phthalate (OEHP) in human placenta which relies entirely on three rapid partition steps prior to gas chromatography with electron capture detection. The use of adsorption chromatography with all its attendant disadvantages for sample clean-up is eliminated in this procedure. Placental samples taken from women who had given birth to a normal baby contained 0.06 ± 0.02 ppm of OEHP. The principal limitation of this method

and of any others, is the high blanks given by laboratory equipment and solvents. The reduction of contamination to workable levels is described.

Determination of Selenium in Biological Samples by Gas-liquid Chromatography with Electron-Capture Detection

Selenium can be determined quantitatively in biological samples after nitric acid-magnesium nitrate digestion and formation of 5-nitropiaz-selenole, by extraction into toluene for gas-liquid chromatography with electron-capture detection. The method is suitable for the determination of selenium in orchard leaves, bovine liver and human placenta, hair, blood and urine. Placental samples from normal births contained 0.23 - 0.48 $\mu\text{g/g}$ of Selenium.

Lead Levels in Human Placentae from Normal and Malformed Births

Placental lead levels were studied in a series of Birmingham births classified by stillbirth, neonatal death, or survival beyond one week. There was an appreciable range of lead levels even in normal births (0.15-3.56 $\mu\text{g/g}$) but nevertheless average results showed a marked excess of lead in those who failed to survive both birth and the neonatal period of table. There was no association of placental lead with impaired birthweight among survivors but, in common with other authors, we noted a seasonal variation. The placentae from Indian women had similar lead levels to those from European women and lower values were found in the normal siblings of stillbirths and neonatal deaths (cf Table.)

In preliminary work which we have carried out on placental specimens from Stoke-on-Trent where some of the mothers were working in the pottery industry no high values were obtained and mean values were only half those found in similar Birmingham samples.

If substantiated by further work, our findings concerning placental lead levels in stillbirths and neonatal births are remarkable. In only 7% of the normal births were placental lead levels greater than 1.5 $\mu\text{g/g}$ whereas 61% of the stillbirths or neonatal deaths had levels greater than this. This does not of course mean that lead must be a causal factor in such deaths but it certainly suggests the necessity for further lead analyses on stillbirth tissues. The alternative explanation, that lead accumulates in times of fetal stress, is one which the present evidence would appear to support. For an assessment of this proposal we have planned further work which will include in addition the analyses of maternal tissues or fluids.

Table

NATURE OF SAMPLES	Pb μg/g fresh wt. basis
NORMAL INDIAN SAMPLES	1.13±0.68
EUROPEAN SAMPLES FROM	
(a) NORMAL BIRTHS	0.93±0.64
(b) NORMAL PREMATURE BIRTHS	0.96±0.28
(c) MALFORMED STILLBIRTHS AND NEONATAL DEATHS	1.49±0.69
(d) NEONATAL DEATHS	1.73±0.57
(e) STILLBIRTHS	1.45±0.50
INDIAN AND EUROPEAN SIBS SAMPLES	0.75±0.19

Contractor: British Food Manufacturing Industries Research
Association

Contract no 058 - 74 - 9 ENV. UK

Project leader: Dr. C.L. Walters

Title of project: "The Formation of N-Nitroso Compounds of
Biological Importance from Nitrite and Nitrate in the Environment."

Nitrosamine precursors in environmental sources:

Since complex molecules can be degraded by nitrite to yield simple nitrosamines, the examination for precursors to N-nitroso compounds has proceeded by reacting them with relatively high concentrations of nitrite under optimum conditions of pH. Subsequently, the N-nitroso compounds formed have been fractionated on the basis of their volatility in air, extractability etc.

Using a group specific method (Eisenbrand & Preussmann, 1970) for the detection of volatile nitrosamines arising from reaction with nitrite at pH 3.0, volatile nitrosamines were obtained from potatoes, carrots, Brussels sprouts and swedes. By gas chromatography coupled with high resolution mass spectrometry, individual components of the mixtures of nitrosamines such as N-nitrosopiperidine and -pyrrolidine have been characterized and quantified. However, the sum of the concentrations of the simple dialkyl and heterocyclic nitrosamines usually detected in biological systems has been consistently far less than the total contents as determined by the group specific procedure, indicating that other unusual nitrosamines were present which remain as yet uncharacterized. Non-volatile N-nitroso derivatives have also been obtained from the reaction of precursors in carrots and one major fraction present to the extent of about 50 μ moles per kg original material has been purified to a single nitrosamine component in chromatography. It has a nitrogen content of about nine atoms per N-nitroso group i.e. equivalent to seven further nitrogen atoms unrelated to the $\text{>N} - \text{NO}$ group. Its stability to acid and alkali precludes its being a N-nitroso derivative of the type obtained from a simple peptide such as glycylglycine not containing a conventional secondary amine group.

Ziram added to carrots at a level of 100 p.p.m. prior to their reaction with nitrous acid at pH 3.0 led to a commensurate increase of volatile nitrosamines due to the formation of N-nitrosodimethylamine. Hence, studies have been carried out at the natural pH values of known cultivars of spinach, peas, potatoes, beetroots, carrots, Brussels sprouts, raspberries and damsons at a nitrite concentration of 1000 p.p.m. All commodities contained nitrate, the maximum value being 317 p.p.m. KNO_3 in spinach but beetroots were the only one with an appreciable content of nitrite (22 p.p.m. NaNO_2). Of the commodities giving rise to detectable volatile nitrosamine, raspberries had a natural pH of 2.9 close to the optimum values for nitrosation but both carrots (pH 5.98) and potatoes (pH 5.73) also gave a positive response. Thus it would appear that some vegetables contain factors conducive to nitrosation at pH values at which chemical reaction with nitrous acid in simple aqueous solution can occur but not readily.

The involvement of plant nitrate reductases in the nitrosation of amines: Nitrate and nitrite reductase systems occur widely in plants and could participate, therefore, in the nitrosation of amines at pH values within the 'environmental' range. With the collaboration of Dr. B. Notton of the Long Ashton Research Station, University of Bristol, samples of spinach nitrate reductase have been purified to high activities using either reduced nicotinamideadenine dinucleotide (NADH) or reduced methylviologen as cofactors. The optimum pH of the enzyme preparations was 7.4; little activity remained below pH 4.6 but 20% of its capacity to reduce nitrate to nitrite was retained at pH 5.5, where some chemical nitrosation would be anticipated, particularly of the less basic amines.

Nitrate reductase preparations have been incubated at pH values in the range 3.0 - 7.5 with nitrate, NADH and the amines morpholine, proline and hydroxyproline. So far, no additional N-nitroso compounds have been extracted as a result of synthesis by the enzyme action; the maximum concentration of nitrite so far achieved as a result of enzyme action is 4.5 mM, which compares with the legal limit of 2.9 mM which applies to its use as a preservative in foods in many countries.

Analysis of N-nitroso compounds

a. Volatile compounds

A procedure has been developed for the unambiguous characterization and estimation of volatile nitrosamines using a Perkin Elmer F11 gas chromatograph coupled via a Watson-Bieman interface with a Hitachi-Perkin Elmer RMU 7L double focussing mass spectrometer.

At a resolution of 10,000 (30% valley), the mass spectrometer is focussed precisely on the parent molecular ion of each nitrosamine using a noncarcinogenic isomer such as cyclopropane carboxylic acid hydrazide for N-nitrosopyrrolidine. The limit of detection is approximately 1 ng in the volume injected, with the exception of N-nitrosodipropylamine, for which a higher detection limit applies.

b. Non-volatile compounds

Since no general procedure akin to that for volatile compounds is available for the separation and concentration of non-volatile nitrosamines and nitrosamides, a procedure is under development whereby such compounds can be estimated directly as a group on a freeze-dried biological matrix without preliminary extraction.

In kinetic studies, the most effective denitrosating agent of a nitrosamine has proved to be hydrogen bromide which has therefore been employed in conjunction with a modified British Oxygen 101A Luminox chemiluminescent analyzer. This analyzer is constructed to detect nitric oxide at low levels, its action being based on the formation in the presence of ozone of activated nitrogen dioxide which relaxes to its ground state with accompanying emission of light in the far visible and near infrared regions. After denitrosation with hydrogen bromide, the volatile product nitrosyl bromide has been conveyed through a converter designed to remove the halogen, into the analyzer which has been constructed in teflon to eliminate corrosion problems.

N-nitrososarcosine has been synthesized as a typical non-volatile nitrosamine of biological origin. In simple solution, a linear response from it has been obtained using the chemiluminescent analyzer over at least the range 5.7 ng to 200 μg i.e. the method is far more sensitive than the group specific procedure of Eisenbrand & Preussman (1970). Furthermore, the repeatability and reproducibility of the chemiluminescent analyzer procedure were at least as good as those using the alternative technique. Compounds which interfered in both methods included inorganic nitrite, alkyl nitrites and S-nitrosothiols. Evidence has already been obtained, however, that the use of acetic acid alone prior to denitrosation with hydrogen bromide in the same solvent leads to the successful differentiation between such compounds and N-nitrososarcosine.

Factors in the human stomach potentially influencing the nitrosation of food amines:

Thiocyanate, which occurs naturally in the saliva, has been found to catalyze the nitrosation of secondary amines in simple solution by up to several hundred fold (Boylard & Walker, 1974). The significant three fold increase in the salivary thiocyanate level of smokers over that of non-smokers has been paralleled by a similar proportional and significant difference between the two groups in gastric thiocyanate, the overall mean values being 1.5 and 0.48 mM respectively. No similar significant difference has been found for total gastric phenols.

N-nitroso compounds arising from incubations of foods in vitro and in vivo:

The formation from food amines of N-nitrosopiperidine (NO_{Pip}) and -pyrrolidine (NO_{Pyr}) during incubation in vitro at acid pH with a nitrite level (0.46 mM) likely to be encountered in the human stomach is dependent in large measure on the concentration of thiocyanate within the range 0 - 3.0 mM.

With the collaboration of a teaching hospital, homogenates of normal foods including those prepared using nitrite have been introduced into the stomachs of volunteers and recovered after residence times of thirty minutes. In three instances out of six, very small amounts of N-nitrosopiperidine were detected by gas chromatography coupled with high resolution mass spectrometry in the products recovered, along with non-volatile nitrosamines particularly in the non-dichloromethane extractable fraction, as detected by a group specific denitrosation procedure.

Nitrite levels in gastric juice and their response to dietary intake:

The ingestion of meals containing luncheon meat to provide nitrite to an overall level of 0.83 mM by a number of student volunteers was accompanied by a rise in the mean gastric nitrite level to almost 0.3 mM concurrently with a rise in gastric pH to about 4.6. Approximately 50 minutes after the commencement of the meal, the gastric pH commenced to decrease, the fall being paralleled by a reduction in the nitrite level to about 0.15 mM at pH 3.2 some thirty minutes later; thus the nitrite which was found to disappear either reacted with gastric components at the lower pH values then occurring or was absorbed or passed on through the pyloric sphincter into the duodenum. Salivary nitrite levels showed a small fall on average after the meal, presumably due to dilution, and then recovered again within about 60 minutes.

After removal of residual resting gastric juice, pentagastrin stimulation promotes the secretion into the stomach of hydrochloric acid to the extent of a five or more fold increase in volume. Over a period of up to 30 minutes, large and significant falls in gastric thiocyanate and total phenol levels were observed in eight patients subjected to pentagastrin stimulation coincidentally with a fall in pH, whereas the gastric nitrite levels remained unchanged. This suggests that nitrite, unlike thiocyanate, enters the stomach concurrently with hydrochloric acid during pentagastrin stimulation.

The highest levels of gastric nitrite were observed in two cases of achlorhydria with concentrations of 51 and 110 μ M accompanying pH values of 7.4 and 7.3 respectively.

References:

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Eisenbrand, G. & Preussmann, R. (1970) Arzneimittel Forsch, 20: 1513.

Contractor: University of Sussex, Falmer Brighton

Contract no: 089-74-10 Env. U.K.

Project Leaders: C. F. Arlett and R. J. Cole

Title of Project: Evaluation of the mouse granulocyte-macrophage colony forming system as a method for mutation assay.

- A. The lack of a mammalian host-mediated assay system which allowed measurement of (a) mutagenic events occurring in vivo by analysis of primary clones in vitro and (b) effects of long term exposure to mutagenic agents, was identified as a serious gap in the range of available techniques for assessing the DNA damaging potential of environmental pollutants. The basic properties of existing methods for cloning haemopoietic progenitor cells, especially the granulocyte/macrophage colony forming cell (CFUc) suggested that this system could be developed to form such an assay. CFUc are progenitor cells, direct descendants of multi-potent haemopoietic stem cells. They occur in significant numbers in rodent foetal liver, and post-natal bone marrow, spleen and peripheral blood. CFUc may also be obtained from human bone marrow and peripheral blood.

Maximal cloning efficiency of rodent CFUc in vitro depends on a growth regulating factor, 'colony stimulating activity', and a promotor isolated from erythrocyte membranes. Optimal conditions for clonal growth were established using LS cell conditioned medium as a source of colony stimulating activity and promoting activity from rat erythrocyte preparations. Bone marrow from normal mice (FL4/Re male) yields approximately 3×10^4 colonies per 10^6 nucleated cells, and this can be enhanced by stimulation with adjuvant or endotoxin. Thus, one adult mouse can provide approximately 2×10^5 CFUc from 2 femurs.

In the first stage of the development of this system as a potential mutation assay, the toxicity of 4 selective agents

(indications of mutations at specific loci), ouabain, thioguanine cytosine arabinoside and db cyclic AMP/theophylline, and one model mutagen, ethyl methanesulphonate, were determined.

Preliminary estimates of the spontaneous and induced mutation frequency to ouabain and thioguanine resistance were established and some problems in the evaluation of these results were identified, e.g. cell concentration effects, in vivo expression time, and inhibition of 6-thioguanine toxicity by growth promoting agents. The frequency of CFUc showing spontaneous resistance to 10^{-3} M ouabain was estimated at $< 1 \times 10^{-6}$, and to 6-thioguanine (1.8×10^{-4} M) $\leq 6 \times 10^{-5}$.

Further improvement could be achieved by interposing a period of mass culture of cells in liquid medium between in vivo exposure to mutagen in in vitro cloning in selective medium. Techniques yielding 5.5×10^4 CFUc per 10^6 original marrow cell input for 8 days in liquid medium have been established.

B. Comparative studies with L5178Y mouse lymphoma cells.

(1) A comparison of ouabain, thioguanine and excess thymidine resistance as selective agents with EMS as a model mutagen [1]

We have been able to establish toxic levels for these compounds and describe the effect of expression time upon the recovery of resistant variants. The data obtained indicate that ouabain is a valuable alternative selective agent to thioguanine which has been traditionally used in such experiments. Equal EMS treatments yielded substantially more variants resistant to thioguanine than to ouabain. It is suggested that this difference depends on constraints in the classes of mutants which are permissible in a vital function, maintenance of the Na^+/K^+ balance, when compared with a non-vital function, salvage purine biosynthesis. Such specificity may lead to a classification of the specific action of mutagens.

(2) Methyl methane sulphonate mutagenesis in L5178Y cells [2]

The effect of long (20h) and short (2h) treatment of L5178Y cells with methyl methane sulphonate was examined. The mutagen was

found to be both cytotoxic and growth inhibitory. A dose dependent induction of thioguanine- and thymidine- but not ouabain-resistant variants was observed. A comparison of these results with those obtained with EMS showed that MMS on a molar basis was approximately ten times more toxic than EMS. With mutation, however, when evaluated at equal levels of cell killing MMS and EMS induced the same number of thymidine-resistant variants. For thioguanine-resistant variants MMS was approximately 10-fold less efficient than EMS, while for ouabain-resistance MMS, unlike EMS, produced no variants at all. It is suggested that some reasons for these differences may include the nature of the mutagen and the selective agents. The ouabain results were further compared with the positive results obtained using a modified Luria-Delbrück fluctuation test.

(3) Fluctuation tests with ouabain as the selective agent [3]

A modified version of the classical Luria-Delbrück fluctuation test has proved possible using ouabain as a selective agent. The spontaneous mutation rate using this method is within the range $0.44 - 1.03 \times 10^{-7}$ mutants per cell per generation. Addition of very low non-toxic levels of EMS (0.1 mM) and MMS (0.012 mM) gave a highly significant increase in the number of ouabain-resistant mutants. It is suggested that the modified fluctuation test is a simpler and considerably more sensitive assay for mutagens than the conventional experimental design.

Conclusions

Our evaluation indicates that the mouse granulocyte-macrophage (CFUc) cloning procedure might be developed to provide a valuable "host-mediated" mutation assay, overcoming many of the disadvantages inherent in current assays of this type. The sensitivity of detection of the effects of low levels of mutagen could be enhanced by a "fluctuation test" type analysis, using individual femurs as unit replicates. It appears that in the continued development of this system, attention should be paid to the kinetic state of the mutable "target cells" and their position in haemopoietic cell lineages.

The L5178Y mouse lymphoma system provided useful model experiments in the evaluation of a range of selective agents and, in particular, in the development of experimental design in the form of a modified fluctuation test which leads to substantial increases in the sensitivity of testing. Both these properties are capable of further exploitation and there is the possibility of further development by the use of co-cultivation with a metabolically active cell type.

Publications

1. Cole, J. and Arlett, C.F. Ethyl methanesulphonate mutagenesis with L5178Y mouse lymphoma cells; a comparison of ouabain, thioguanine and excess thymidine resistance. *Mutation Res.*, 34, (1976) 507-526.
2. Cole, J. and Arlett, C.F. Methyl methanesulphonate mutagenesis in L5178Y mouse lymphoma cells. *Mutation Res.*, in press.
3. Cole, J., Arlett, C.F. and Green, M.H.L. The fluctuation test as a more sensitive system for determining induced mutation in L5178Y mouse lymphoma cells. *Mutation Res.*, 41 (1976) 377-386.

Contractor : Department of Genetics, Trinity College
University of Dublin

Contract n° : 023-74-1 ENV EIR

Project Leader : G.W.P. Dawson

Title of Project : The establishment of a convenient test for somatic
mutations in mammals

In 1957 L.B. Russell and M.G. Major published (Genetics, 42, 161-175) an account of the induction of presumed somatic mutations in the house mouse by radiations. A strain (the T strain) which was homozygous recessive for 7 factors was crossed with a wild type strain. The embryos were irradiated by exposing the pregnant females to X-rays and a mutation induced in the wild type allele of one of the 7 factors would establish a cell line which would be homozygous recessive for that factor. If this is one which affects coat colour, and the colour of any region of the coat is determined by the genotype for that factor of the cells of that region, a spot of mutant coat colour will occur. Mutations in 5 of the 7 factors are probably scorable in this way.

Apart from the paper in 1957 no further work was done to either develop or use this system until the present project. Following a visit of Professor Dawson to the laboratories of the Atomic Energy Commission at Oak Ridge, Tennessee, to discuss with Dr. Russell the reasonableness of our working on the development of the system, and gaining her full support, we substituted a different test cross to increase the sensitivity of the test and developed suitable procedures for using it to detect the mutagenicity of chemicals.

Female mice of the T strain (non-agouti, aa; pink eye, pp; brown, bb; dilute, dd; chinchilla, $c^{ch}c^{ch}$; spotted, ss; short ear, sese) are mated to males of the HT strain - a strain developed at the Atomic Energy Research Establishment, Harwell, U.K. - (non-agouti, aa; leaden, llnl; pearl, pepe; pallid, papa; fuzzy, fzfz; brachypody, bpbp). This cross enables mutation at pink eye (which also affects coat colour), pallid, chinchilla, leaden,

dilute and brown to be easily seen; mutant areas of homozygous pearl are less easily scored against the normal background coat colour of the offspring and mutations of fuzzy have not been seen.

After establishing the toxicity of the chemical to be used and deducing the maximum dose which can be given, treatment is sometimes orally, but usually intraperitoneal injection. Full experimental details have been given in the first publication of results (G.E. Davidson and G.W.P. Dawson, *Mutation Research*, 38, 151-154, 1976).

The results have shown that the test is sensitive and likely to be useful in recognising the mutagenicity of some chemicals. So far the following chemicals have been used: benzo(a)pyrene, natulan, methylmethane sulphonate (MMS), ethyl methylsulphonate (EMS), dimethylnitrosamine (DMNA), diethylnitrosamine (DNA), urethane, mitomycin C and amitrole. There is at present clear evidence from this test system of the mutagenicity of benzo(a)-pyrene, natulan, MMS, mitomycin C and urethane. Work on studies of the effects of the other compounds are either not complete or are being re-examined using different doses. These studies have also revealed that it may be important to distinguish between white-spotting, which may be due to the death of cells, and non-white spots. We already know that the time when treatment is given can be critical; around the 9th day of pregnancy being often the most sensitive time.

The test is speedy (offspring can be examined 21 days after birth) and sometimes only about 20 litters (about 150 offspring) are necessary to reveal the mutagenic activity of a chemical.

Development of the Microbial Assay for screening
environmental pollutants.

The overall objective of this research has been to develop a test system in bacteria which could detect potential mutagenic and carcinogenic agents more reliably than possible previously. Since the initiation of this research the most thoroughly examined test system combining simplicity and economy as well as speed and reliability has undoubtedly been the Ames Salmonella system. The Ames Test however fails to detect some carcinogens as mutagens. Our objective has been to pursue several lines of investigations aimed at overcoming this deficiency of the microbial system. These lines have been:

- (1) The development of an alternative test system exploiting forward mutagenesis in contrast to the Ames back-mutation system.
- (2) Isolation of other testers with further specific targets at present absent from the set of Ames Testers.
- (3) Parallel in vitro and in vivo mutagenicity tests to establish whether part of the deficiency of the Ames system is due to tissue specificity in the activation of compounds to mutagens.

Our investigations on one forward mutagenesis system, the galactose-resistance system, are now completed. This system has enabled us to detect the carcinogens thiourea, thioacetamide, amitrole and succinic anhydride as mutagens, none of which are detectable by the Ames system (manuscript in preparation for publication). In brief, in this system resistance to galactose can arise in a population of previously sensitized cells, if either of two genes of the galactose operon is inactivated through mutations. Unlike other forward mutagenesis systems such as streptomycin resistance or rifampicin resistance which restrict the scorable mutations to base-substitutions, this system allows base-substitutions and frameshift mutations as well as extended deletions to be scored. We first showed that the forward mutagenesis system originally proposed by Professor Ames, the azetidine carboxylic acid resistance

system, is not a utilizable system as it does not respond to mutagens such as 9-amino acridine, 2,7-diaminofluorene or 2-nitrosofluorene which have a high affinity for cytosine-guanine repeat sequences in DNA. In contrast the galactose-resistance system responds to such mutagens and also to further mutagens chosen to demonstrate that the system contains all the sequences present in the four specific testers of Ames. The disadvantage of this system for general use is its relatively high spontaneous mutation rate under some conditions. For example, in the absence of liver homogenate activating system, the spontaneous rate can be regulated by controlling the levels of galactose and other mineral salts in the medium. However, presence of glucose resulting from conversion of hepatic glycogen in crude liver homogenate preparations partially relieves the sensitivity to galactose resulting in a high spontaneous rate to resistance. This glucose effect can be overcome by the preparation of pure microsomes by Ca^{++} precipitation technique, but unfortunately microsomal enzyme levels are then relatively poor! A compromise can be reached by preparation of crude liver homogenates from animals fasted for 36 hours. As expected, the introduction of repair deficiency mutations such as uvrB^- and PolA^- , or the introduction of PKM101 R factor carrying a recombination repair deficiency raise the spontaneous rate of mutation. The levels are acceptable in uvrB^- or PolA^- derivatives, but in the presence of R factor plasmid, the spontaneous rate would probably mask the mutagenic effect of many relatively weak mutagens. Thus although the system contains all the already identified targets, responds to a wide range of mutagens and is capable of detecting some mutagens undetectable by the Ames Testers, we consider it unsuitable for general distribution and are proceeding with the systematic examination of alternative forward mutagenesis systems.

In our programme of work initiated in January 1975 on the protein sequencing of trpA gene product in Salmonella for the construction of testers with new mutagen specific sequences, several interim targets have now been completed. The wild-type trpA gene and two pseudo-wild-type strains to be

used for peptide fingerprinting and base-sequence identification, have been constructed by suitable genetic manipulations for 100 litre batch preparations for enzyme extracts. This has involved the insertion of the *trpA* region carrying the relevant mutations into strains carrying a late enzyme block in tryptophan manufacture so that the operon could be maximally derepressed for the enzyme of the system. Recombinant plasmids have been constructed carrying both the wild-type and pseudo-wild-type *trpA* genes and introduced into each parental strain in order to have multiple copies of the *trpA* gene in each strain. The enzyme from the wild-type *trpA* gene has been purified by steps including gel filtration and ion exchange chromatography and currently the enzyme from the pseudo-wild-types are being purified.

In the parallel in vitro and in vivo programme of mutagenicity tests, a large number of chemicals have now been tested including methyl methane-sulphate, diethylnitrosamine and natulan as part of an E.E.C. group coordinated series of experiments. Several results merit special interest. For example:

(1) 6-chloropurine, an antileukaemic base-analogue, has to date been the only chemical which is mutagenic in vivo but non-mutagenic in vitro in presence of liver microsomes, which may be due to tissue specific requirement for activation.

(2) None of the chemicals previously identified as carcinogens and found non-mutagenic in vitro in the Ames Test show any mutagenicity in the host-mediated assay. These include natulan, thiourea, urethane and amitrole. In the case of diethylnitrosamine, its mutagenicity can be demonstrated in vitro, but not in vivo. These results argue against the usefulness of the host-mediated assay for general screening purposes.

(3) The mutagenicity of MMS, a straight alkylating agent is increased in the presence of microsomal enzymes. Although MMS is an extremely widely used chemical, this result has not been previously documented (manuscript in preparation).

(4) For many chemicals the optimal level of mutagenicity is obtained in Testers which have a normalexcision repair capacity and a defective recombination

repair capacity. This is striking in the case of MMS (Manuscript in preparation for publication) and shown by many related nitrobenzofuroxan derivatives tested by us (Mutation Res. 48, 145, 1977). Currently these studies are being extended to a set of twelve bis-thiosemicarbazones and twenty five related 9-amino acridine derivatives.

(5) We have preliminary evidence that para-rosaniline, a carcinogen previously classified as non-mutagenic in the Salmonella system, is in fact mutagenic in presence of mouse liver microsomes. The documented evidence on the chemistry of what constitutes "para-rosaniline" is confused and complex and we are currently compiling data on this important carcinogenic dye.

Publications

S. Thompson and L. Kellicutt.
Mutagenicity of anti-cancer nitrobenzofuroxans.
Mutation Res., 48, 145-154 (1977)

Contractor : University of Odense (previous residence of project leader)
 Contract n° : 025-74-1 ENV DK
 Project leader : Prof. Jørgen Clausen, Department of Biochemistry and Toxicology, Institute of Life Science, Roskilde University, Chief of Neurochemical Institute, Copenhagen
 Title of project: The content of Polychlorinated Hydrocarbons in Arctic Ecosystems

The purpose of the present study has been to elucidate the content of polychlorinated organic components in the Greenlandish food chains and compare accumulation in aquatic and terrestrial animals of Greenland with those of the human populations in the corresponding areas. Furthermore the purpose has been to compare the content of polychlorinated hydrocarbons in Greenlanders with the corresponding concentrations in the southern Danes. The content of polychlorinated organic components has been elucidated in adipose tissue from the following animals: 1: Mammals: 2 common porpoises (*Phocaena phocaena*), 5 Bearded seals (*Erignatus barbatus*), 5 Ringed seals (*Phoca hispida*), 5 Hooded seals (*Cystophora cristata*), 2 Arctic Foxes (*Alopex lagopus*), 1 Polar Bear (*Ursus Maritimus*) and 1 Sheep (*Ovis aries*). 2: Arctic birds: 3 King Eiders (*Somateria spectabilis*), 1 Common Eider (*Somateria mollissima*), 5 Harlequin Ducks (*Histrionicus histrionicus*), 3 Long Tailed Ducks (*Clangula hyemalis*), 1 Purple Sandpiper (*Calidris maritima*), 5 Brünnich's Guillemots (*Urea lomvia*), 3 Cormorants (*Phalacrocorax carbo*), 5 Ptarmigans (*Lagopus mutus*) 3 Ravens (*Corvus corax*). 3: 35 Eskimos.

The data were furthermore compared to: 4:16 Southern Danes. All arctic animal samples were collected from animals shot on the West Coast of Greenland (from Narssarsuaq in South to Sukkertoppen in North). The adipose tissue samples were mainly taken from the back, sealed in glass tubes and air-mailed to Copenhagen for assay.

The polychlorinated organic components were extracted together with lipids by means of n-hexane. The lipids were separated from the polychlorinated organic components by column chromatography on aluminium oxide (for details see: Clausen, Bræstrup and Berg, 1974).

Among the birds showing the lowest mean concentration of "raw pp'DDE" were the King Eider (mean: 1.7 ppm), The Common Eider (0.8 ppm), The Harlequin Duck (1.1 ppm), and the Purple Sandpiper (1.1 ppm). However, the content was higher in Brünnich's Guillemot (3.5 ppm), while the highest concentrations were

found in the Cormorant (10.3 ppm) and the Raven (13.9 ppm). However, in these animals great individual variation occurred.

In the case of PCB, the lowest concentration (mean values) was again found in the group of ducks, the figures ranging from 3.3 ppm for the King Eider, 2.0 ppm for the Common Eider, 1.2 ppm for the Harlequin Duck and 1.7 for the Long Tailed Duck. In this group, the Purple Sandpiper (2.8 ppm) may also be included. For the other species, the PCB concentration showed a higher concentration, the Ptarmigan containing 10.1 ppm, Brünnich's Guillemot 12.9 ppm, the Cormorant 26.3 ppm and lastly, the Raven 37.1 ppm, but great individual variation occurs.

pp-DDT and pp-DDD were not traced. Dieldrin was probably present in minor amounts, although no attempt was made in the present assay to determine the exact figure since the peak coexisted together with the birds, trace amounts of lindane were found. Correlation analysis (Fig. 1) (Spearman Rank test) revealed a statistically significant correlation in the birds studied between the DDE content and the PCB content (coefficient of correlation 0.9, $p \leq 0.1\%$).

The content of polychlorinated organic components of arctic mammals, Eskimoes and Southern Danes are presented in Table 1. From the Table it can be seen that Eskimoes contain a lower content of lindane and aldrin but a higher content of heptachlor epoxide, pp-DDE and PCB than the aquatic carnivorous. On the other hand the Greenlanders, compared to Southern Danes seem to contain a similar amount of lindane, heptachlor and dieldrin. On the other hand the content of DDT and PCB seems to be higher in the Eskimoes than in the Southern Danes, however Wilcoxon's test for differences between median values in the two groups could not reveal any significant difference.

Only a rough non-significant correlation between age and the residue level of DDT and its metabolites was found in human adipose tissue being different as in Southern Danes and Eskimoes. Greenlanders showed a tendency of a maximum load to occur about the age of 38 years (Table 2). The maximum residue level of Σ DDT in Danes seems to occur somewhat later in lifetime.

The maximum residue level of PCH's in Eskimoes seems to occur about 10-15 years later in lifetime than the maximum level of Σ DDT, but 10-15 years earlier than the maximum level of PCH in Southern Danes' lifetime. The maximal exposition to both polychlorinated components may have occurred years later in Greenland than in Denmark.

Table 2.

Relationship between the age of adult Greenlanders and the mean adipose content of DDT, Aldrin, Dieldrin and PCB.

Age Group	DDT ppm (mean)	PCB ppm (mean)	Aldrin ppm(mean)	Dieldrin ppm (mean)
22-44 (17 observations)	6,8	3,0	0,09	0,16
45-60 (12 observations)	3,1	8,0	0,11	0,14
61-75 (3 observations)	3,5	3,3	0,19	0,09

Table 1.
 CONTENT OF POLYCHLORINATED HYDROCARBONS IN FAT OF SOME IMPORTANT MAMMALS (ppm)

	Lindane	Heptachlor	Aldrin	Heptachlor epoxide	pp'DDE	Dieldrin	op'DDT	pp'DDT	ΣDDT	PCB
Greenlanders (N = 35) Mean ± S.D.	0.01±0.02	0.02±0.05	0.10±0.10	0.15±0.15	3.5±4.2	0.11±0.08	0.6±1.6	0.8±0.6	5.3±6.5	5.5±5.1
Median	0.00	0.01	0.12	0.12	2.0	0.09	0.0	0.8	3.3	3.8
10% decile	0.00	0.00	0.02	0.00	0.4	0.02	0.0	0.2	0.6	1.1
90% decile	0.04	0.03	0.22	0.33	9.2	0.16	1.4	1.7	10.9	14.5
South Danes (N = 17) Mean±SD	0.01±0.02	0.01±0.02	0.21±0.22	0.06±0.05	1.4±0.9 ^Δ	0.12±0.13	0.0±0.0	0.4±0.3 ^Δ	1.9±1.1 ^Δ	3.3±1.0
Median	0.00	0.00	0.08	0.08	1.7	0.09	0.0	0.3	1.8	3.5
10% decile	0.00	0.00	0.08	0.08	1.7	0.09	0.0	0.3	1.8	3.5
90% decile	0.05	0.03	0.53	0.12	2.2	0.14	0.0	0.9	2.7	4.6
Aquatic carnivorous (seal sp. N = 20) Mean ± S.D.	0.06±0.12	0.01±0.02	0.22±0.38	0.11±0.13	0.7±0.5 ^Δ					5.1±5.2
Median	0.08	0.00	0.10	0.06	0.7					2.4
10% decile	0.00	0.00	0.03	0.04	0.1					1.0
90% decile	0.14	0.02	0.43	0.27	1.4					8.5
Terrestrial carnivorous Ursus maritimus	n.d.	n.d.	3.01	0.49	1.3					21.0
Alopex lagopus nr. 91	0.11	n.d.	0.24	0.26	1.3					9.5
Alopex lagopus nr. 95	0.02	0.02	0.10	0.25	0.2					12.0
Mean	0.04	0.01	1.11	0.33	0.9					14.2

ppm = mg/kg on fresh weight basis

Σ DDT = (pp'DDE x 1,11) + op'DDT + pp'DDT

Δ mean value significant different from Greenlanders mean value

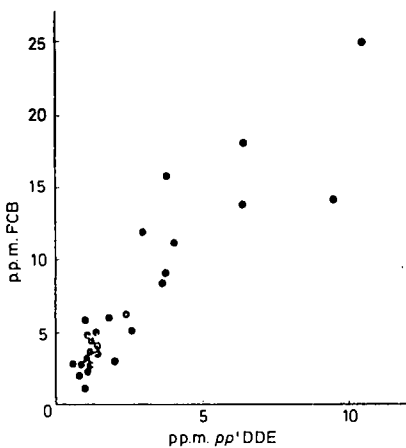


Fig.1.

Litterature:

1. Braestrup, L., Clausen, J. & O. Berg: DDE, PCB and Aldrin levels in Arctic birds of Greenland. Bull. Environm. Cont. Toxicol. 11:326-332, 1974.
2. Idem.: The content of polychlorinated hydrocarbons in arctic mammals. *ibid.* 12:529-534, 1974.
3. The Proc. Pure Appl. Chem. 42:223-232, 1975.
4. Egeskov Jensen, G. & J. Clausen: A comparison of the content of polychlorinated hydrocarbons in Greenlanders and Southern Danes. Dan. Med. Bull. 1977, in press.

Contractor : Bo Holma, Professor, M.D.

Contract n° 026 - 74 - 1 ENV DK

Project leader : Bo Holma

Title of project : Metal Toxicity Analysed by Means of Macrophage
Phagocytosis.

A method for simultaneous evaluation of the phagocytosis of different metal-particles by alveolar macrophages, in vitro, has been developed, and tested. The degree of phagocytosis of different sized particles as well as the toxicity of these can be registered. The method is based on an energy-dispersive X-ray analysis in scanning electron microscopy (SEM) of phagocytosed particles, 1,5 hours after exposure of a known number of macrophages to a known number of particles.

As the degree of phagocytosis among others is dependent of the distance between the particle and the cells (1), the following design of the study was set up.

In one series one macrophage on the average occur per an area of $10000 \mu\text{m}^2$, $250 \mu\text{m}^2$, and $125 \mu\text{m}^2$ respectively. In this way the degree of phagocytosis as a function of particle concentration and the distance between macrophages and particles can be estimated. Each series is further build up so that the macrophages (M) are exposed to one metal, the combination of two or more at the same time.

In the first study exposure is made for metallic copper and vanadium with gold as a reference particle. The following schema is followed in each series.

M + no particles
 M + Au-particles
 M + V-particles
 M + Cu-particles
 M + Au+V-particles
 M + Au+Cu-particles
 M + V+Cu-particles
 M + Au+V+Cu-particles
 M + no particles

One series with one macrophage plus one particle per $10000 \mu\text{m}^2$ is performed. Another experiment with one macrophage plus four particles per $10000 \mu\text{m}^2$ is under way.

The evaluation of the data follows a three dimensional analysis, i.e. 2^3 factorial design.

METHODS

The rabbits used for the experiments (ca. 3,5 kg) are killed by a blow in the neck. Lung lavage in situ is carried out by 3-4 washings by 20 cc of Hank's solution (37°). The lavage fluid is centrifugated at 1500 rpm for 15 minutes and washed once by suspension in Hank's solution followed by centrifugation as above. The cells are then suspended in Hank's together with 20% autolog serum. The cell concentration was regulated by counting in a Bürkers counting chamber, to be approximately $2 \cdot 10^6$ cells/ml. The cells were then transferred by micropipette in quantities of 0,1 ml ($1 \cdot 10^6$ cells) to special cups of polyethylen at 37° . After 20 minutes the supernatants were poured off and $2 \mu\text{m}$ particles of metallic copper, vanadium or gold were added in 1,5 ml in Hank's solution. Control cultures were handled in the same manner except for the metals.

The samples of $2 \mu\text{m}$ were separated from metal powders by sedimentation in a tube with countercurrent alcohol (99%). After collection, the samples were repeatedly washed in alcohol and finally suspended in the Hank's solution immediately before use. The concentration was regulated in the same way as the cells, and with the same concentration.

The cell viability of 200 cells was estimated by light microscopy using the eosin technique and Bürkers counting chamber. Viability tests were performed before and after the period of phagocytosis. After the phagocytosis which were performed during 1,5 hours at 37° and without addition of CO₂, the supernatant was poured off and the cells attached to the surface of the cups were fixed in 4% glutaraldehyde. The cells that did not attach themselves before exposure period as well as after it, were counted and observed to be less than 0,5%.

After fixation the cups were dried at room temperature and a thin layer of carbon was evaporated onto the specimens, under vacuum, to render them conductive for studies in scanning electron microscope (SEM), (Cambridge MK 2). The metal particles phagocytosed by the macrophages were then registered by an energy-dispersive X-ray analyser (EDAX) connected to the SEM.

RESULTS

The data from the 2³ factorial experiment shows that when macrophages are exposed to 2 µm particles (i.e. around the optimal size for phagocytosis) of vanadium, copper or gold as single metals or combinations of these, the gold has no toxic effect but the two others increase the death rate significantly.

Synergistic effects are found, but a fully quantitative evaluation needs further data, were effect of concentration variations can be taken into account. It is, however, indicated that vanadium and copper demonstrate a more than additive effect as regards toxicity.

The technique using the combination of SEM and EDAX gives information of the effect on single cells of different metals or metallic compounds, and different combinations of these which is not possible with other methods.

Contractor : University of Copenhagen
Dept. of Clinical Chemistry
and
M.R.C., St. Bartholomew Hospital, London

Contract n° : 031-74-1 ENV DK

Project leaders : Prof. P. Astrup, Prof. P. Lawther

Project title : Effects of air pollutants on development of
cardiovascular and pulmonary injuries in
experimental animals, with special emphasis
on the occurrence of additive or potential effects

Project no. 1. Additive effects of CO and hypoxia exposure.

Cardiovascular system:

As described in the semiannual progress report of September 1975 we have studied the occurrence of additive effects of carbon monoxide and hypoxia exposure on the development of atherosclerosis in cholesterol fed rabbits. The results indicate that additive effects occur, since the accumulation of cholesterol in aorta was higher in the group exposed to carbon monoxide + hypoxia than in the group exposed to carbon monoxide alone. It should further be noted that exposure to carbon monoxide + hypoxia causes a 40% decrease in the amount of cholesterol fed to the rabbits for maintaining the same serum cholesterol level as in rabbits exposed to carbon monoxide only. This clearly demonstrates an additive effect of CO and hypoxia on cholesterol metabolism, since it is known from exposure experiments performed previously, that exposure of rabbits to CO (200 ppm) or to hypoxia (15% O₂ in atm. air) respectively leads to about the same increase in serum cholesterol.

Respiratory system:

We have studied the effects of CO exposure on the respiratory system of rabbits, because it was necessary to know the

effects, if any, before starting studies on additive effects of CO + hypoxia.

Altogether 24 rabbits were used in the experiment. Continuous CO-exposure (200 ppm) was performed on rabbits exposed for 3 weeks (3 rabbits), 4 weeks (3 rabbits) and 6 weeks (2 rabbits) respectively. Correspondingly intermittent CO-exposure (200 ppm 12 hours daily) was performed on 8 rabbits for 3, 4 and 6 weeks respectively. 8 rabbits served as controls. The techniques for fixating the respiratory tissues for microscopic evaluation was the same as described in the enclosed manuscript describing lung changes after NO-exposure (project 2). The evaluation was done blindly.

There was no significant differences between the 3 groups of rabbits and the controls, so it is concluded tha CO-exposure of rabbits under the used experimental conditions did not lead to changes in the lungs. The results will be published and a manuscript is under preparation. A copy of this manuscript will later on be sent to the Commission.

Studies of additive effects of CO and hypoxia on the respiratory system of rabbits was not performed due to the lack of effects of CO-exposure.

Project no. 2. NO-exposure.

Respiratory system:

Enclosed please find a manuscript entitled: 'Ultrastructural changes of the rabbit lung after a 5 ppm nitric oxide exposure', which has been submitted for publication in 'Ar-

chives of Environmental Health'. The study demonstrated an effect of NO-exposure on the lung, also when evaluated blindly.

To supplement this study a further experiment was carried out by exposing 6 rabbits to 43 ± 8 ppm NO (with 3.8 ± 1 ppm NO₂) for a week. The pulmonary changes here were qualitatively similar to the changes found after exposure to 5 ppm NO, and appeared more frequently in the exposed than in the non-exposed animals. The difference between the experimental and the control animals was, however, not significant when studied blindly. This might be due to adaptation processes, so we plan to repeat this experiment in September 1977 before drawing any conclusions concerning the pulmonary toxicity of NO-exposure. When this experiment is finished the results of the investigation will be published. A copy of the final manuscript will be forwarded to the Commission.

Atherogenic effects:

The uptake of cholesterol in the aortic was of NO-exposed rabbits (43 ppm NO) did not differ significantly from the uptake in control rabbits. It is therefore concluded that NO in the used concentration did not influence the atherogenic process.

Binding of NO to hemoglobin:

We did not find a binding of NO to hemoglobin in the exposed rabbits.

Project no. 3. Additive effects of NO and CO-exposure.

We studied the morphology of rabbit lung, aortic arch and thoracic aorta following exposure to 200 ppm CO + 5 ppm NO for 2 weeks.

Exposure conditions as earlier described.

NO concentration in chamber air: 4.9 ± 0.3 ppm (SD)

NO₂ - - - - : 0.07 ± 0.02 ppm (SD)

CO - - - - : 190 ± 10 ppm (SD)

1 μ sections for blind light microscopic investigation, prepared as earlier described, were cut from lungs, pulmonary artery, aortic arch and thoracic aorta.

Results are tabulated in Table I - IV:

Table I: Blind light microscopic investigation of lung tissue from rabbits exposed to 200 ppm CO + 5 ppm NO for 2 weeks.

	exposed n=12	control n=12
No. of animals with changes	5	3
- - - without changes	7	9

not significant, n=no. of sections studied.

Table II: Blind light microscopic investigation of pulmonary arteries of rabbits exposed to 200 ppm CO + 5 ppm NO for 2 weeks.

	exposed n=11	control n=11
No. of animals with changes	4	3
- - - without changes	7	8

not significant, n=no. of sections studied.

Table III: Blind light microscopic investigation of aortic arch of rabbits exposed to 200 ppm CO + 5 ppm NO for 2 weeks.

	exposed n=6	control n=6
No. of animals with changes	5	5
- - - without changes	1	1

not significant, n=no. of sections studied.

Table IV: Blind light microscopic investigations of thoracic aortae of rabbits exposed to 200 ppm CO + 5 ppm NO for 2 weeks.

	exposed n=6	control n=6
No. of animals with changes	1	4
- - - without changes	5	2

not significant, n=no. of sections studied.

The morphological changes earlier described in lung vasculature and aortic endothelium following exposure to 200 ppm CO could not be reproduced, when CO was combined with 5 ppm NO. There is presently no explanation for this discrepancy, but investigations are being carried out in order to throw light on this problem.

Conclusions

The conclusions of the experiments performed are as follows.

1: Additive effects of CO and hypoxia exposure on the metabolism of cholesterol have been demonstrated. The results further indicate an enhancement of cholesterol accumulation in the aortic wall.

2: Continuous or intermittent exposure of rabbits to 200 ppm CO does not cause microscopic changes of the lungs.

3: Exposure to 5 ppm NO might cause slight microscopic changes in the lungs, but further experiments (in preparation) are needed for clarifying this finding.

4: Exposure to 5 and 43 ppm NO respectively does not enhance the development of atherosclerosis.

5: Additive effects of NO and CO exposure on the respiratory and cardiovascular systems could not be demonstrated.

TOPIC 4 : ECOLOGICAL EFFECTS OF WATER POLLUTANTS

Contractors: Universität des Saarlandes - Fachbereich 6 Geographie,
Saarbrücken

Contract n° 012-74-1 ENV D

Project Leader : Prof. Dr. P. Müller

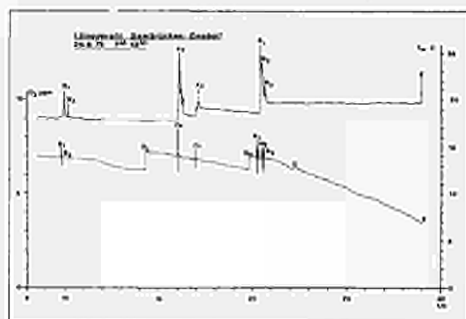
Title of project : Ökologische Kriterien für eine grenzübergreifende
Bewertung der Belastung und Belastbarkeit der Saar

Ziel der Untersuchung

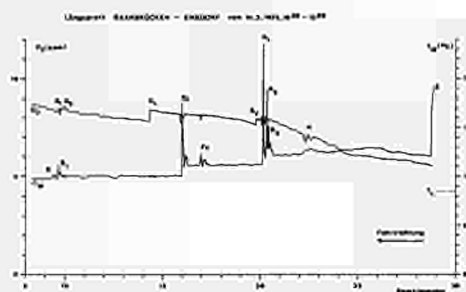
Ziel der gemäß EG-Vertrag Nr. 012-74-1ENV D durchgeführten Untersuchung war die Erstellung eines "Wirkungskatasters" (Vitalitätstests mit an verschiedenen Standorten exponierten Organismen) der in der Praxis für die Bewertung der thermischen und chemischen Belastung der Saar eingesetzt werden kann. In ständiger Zusammenarbeit mit dem Institut Européen d'Ecologie (Metz; Prof. Dr. Pelt, Dr. Pierre), der Fondation Universitaire Luxembourgeoise (Arlon; Prof. Dr. Schmitz) und den für die Wassergüte zuständigen Dienststellen der Bundesrepublik Deutschland (u.a. Wasserwirtschaftsamt für das Saarland; Bundesanstalt für Gewässergüte in Koblenz) wurde dieses Ziel durch chemisch-physikalische Faktorenerfassungen, mehrfache Erfassung und Bewertung von Benthosbiozöosen und durch die Entwicklung von Expositionstests erreicht:

1. Chemisch-physikalische Faktorenerfassung

- 1.1 Es wurden 1974/75 alle Einleiter in die Saar (von Saargemünd bis Konz) kartiert. Diese Erfassung wurde begünstigt durch Niederlegen der Wehre.
- 1.2 Viermal pro Jahr (1974/75) wurden Längs-, Quer- (8 Standorte) und Tiefenprofile (Wehre) der Temperatur in der Saar durchgeführt (korreliert zu Strömungs- und Abflußbestimmungen).

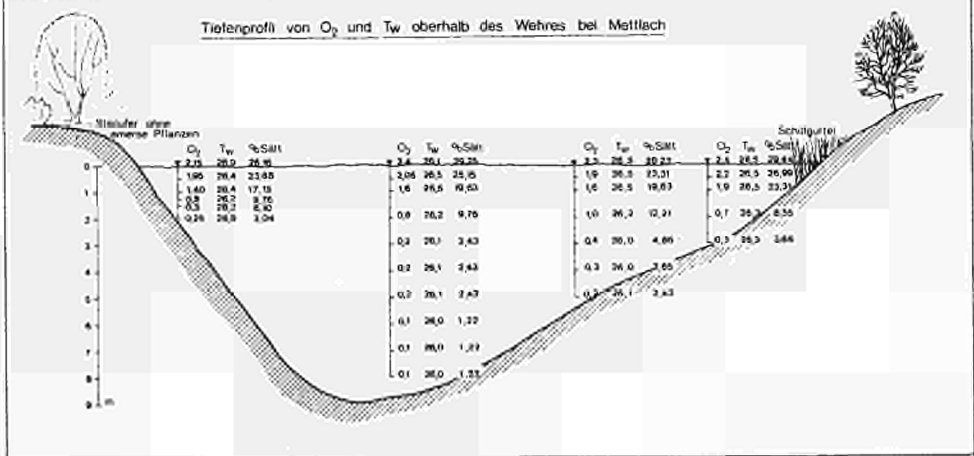


Temperatur- und Sauerstoffkurve der Saar zwischen Flußkilometer 8 (Saarbrücken) und 30 (Ensdorf) bei „Strahlungswehre“ im Juni 1975.



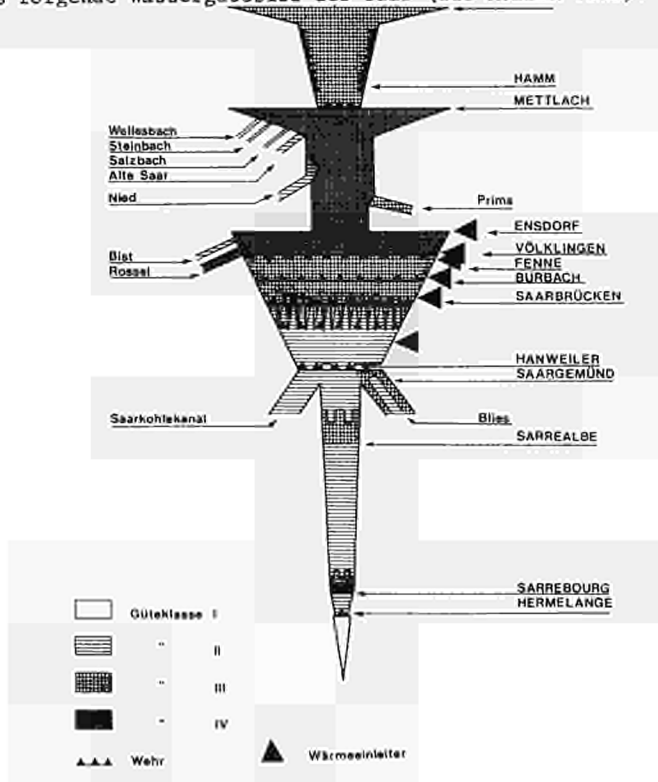
Temperatur- und Sauerstoffkurve der Saar zwischen Flußkilometer 8 (Saarbrücken) und 30 (Ensdorf) während einer Hochwasserituation im März 1975.

Die Oberflächentemperaturen (15 cm Tiefe) nehmen (in Abhängigkeit zu Wasserkörper und Jahreszeit) von Wärmeeinleiter (Kraftwerke, Hütten) sprunghaft zu.



Verstärkt durch sauerstoffzehrende Prozesse sinkt der O_2 -Gehalt ab und erreicht im Bodenbereich von Stauwehren die Nachweisbarkeitsgrenze.

1.3 An den Untersuchungsstellen von 1.2, zusätzlich jedoch an 51 Fundorten von Benthosbiozönosen (vgl. 2.) wurden viermal pro Jahr (1974/75) Schlamm- und Schwebstoffproben sowie 1422 Bestimmungen des Sauerstoffgehaltes, der Sauerstoffsättigung, des BSB_5 , des CSB und der NH_4 -Frachten durchgeführt. Diese Analysen ergeben das folgende Wassergütebild der Saar (aus MÜLLER 1975):



2. Erfassung und Bewertung der Benthosbiozösen



Zwischen chemisch-physikalischer Belastung und den Arealssystemen (vgl. MÜLLER 1977) der Benthosorganismen die 1974 und 1975 an 55 Untersuchungsflächen in der Saar kontrolliert wurden, bestehen enge Korrelationen. Ein markanter Einschnitt ist die Rossel (Fundort 28; km 18,30). Hier kommt es zur völligen Auslöschung der Mollusken- und Crustaceen-Populationen. 22 submerse Wasserpflanzen zeigen an dieser Stelle den größten Artenfehlbetrag.

Die NH_4 -Frachten reichen für sich genommen aus, diesen Einschnitt zu interpretieren. Eine Bewertung aller Benthosorganismen nach dem Saprobienystem (vgl. Deutsche Einheitsverfahren) zeigt, ebenso wie die absolute Zahl submerse Wasserpflanzen, daß sich die Wassergüte der Saar ab der Niedmündung wieder verbessert.

Diversitätsanalysen (vgl. MÜLLER 1976, 1977, MÜLLER und SCHÄFER 1976), die nach verschiedenen mathematischen Modellen durchgeführt wurden, sichern diesen Belastungsgradienten ab. Drei Phänomene waren jedoch besonders auffallend:

1. In ausgedehnten Verödungsstrecken können lokal, bedingt durch eindringendes Grund- und Oberflächenwasser, Biozösen auftreten, die eine wesentlich bessere Wasserqualität anzeigen als der Hauptwasserkörper.
2. Vor allem Fische zeigen außergewöhnliche Adaptationsfähigkeiten. Bei ausreichender O_2 -Versorgung dringen sie auch in für Gastropoden toxisches Milieu vor. Die Auswirkungen dieses Eindringens auf die Fertilität werden weiter untersucht.
3. Bei Mollusken können bestimmte Alleltypen unterschiedliche Wasserqualitätsbereiche besiedeln und damit zu Indikatoren verschiedener Wassergüte werden (*Physa acuta*; weitere Untersuchungen sind im Gange).

3. Expositionstests

Die Erfassung und Bewertung der Benthosbiozösen wurde in Beziehung gesetzt zur Verweildauer, Vitalität und Schadstoffaufnahme von in Unterwasserboxen exponierten Organismen. Als Versuchstiere dienten besonders *Physa acuta*, *Dreissena polymorpha*, *Unio*-Arten, Karauschen, Goldorfen und Rotfedern. Verschiedene Boxmodelle wurden entwickelt. Die Ergebnisse lieferten drei bemerkenswerte Erkenntnisse:

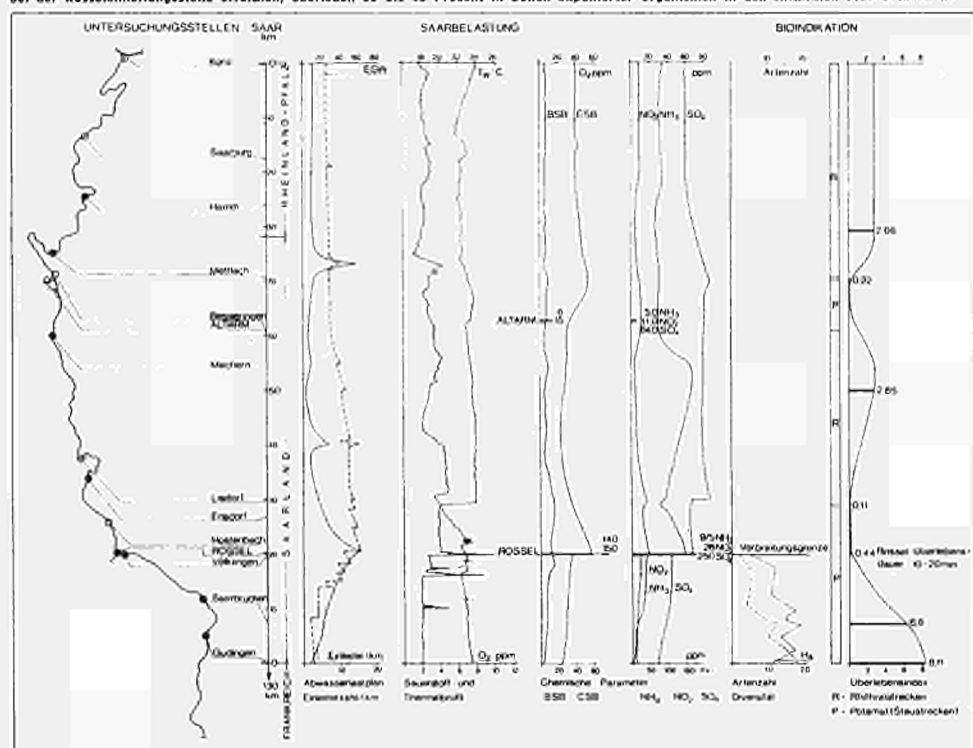
- 3.1. Die Fische zeigen, je nach Eingewöhnungsdauer, eine ausgeprägte Adaptationsfähigkeit. Soweit thermale Einleiter zu keinem Sauerstoffdefizit führen, nimmt die Biomasse mit Expositionszeit und Wassertemperatur zu, die Produktivität jedoch ab (weitere Untersuchungen über Gonadenreife sind im Gange).
- 3.2. *Physa acuta*, *Dreissena polymorpha* und *Unio tumidus* bewährten sich als hervorragende Expositionsarten. Sie sind in ausreichender Zahl von einer Ausgangspopulation zu beschaffen, besitzen unterschiedliche ökologische Zeigerqualitäten und reichern, in Abhängigkeit von der Salzkonzentration ihres Milieus,

Schwermetalle und andere Schadstoffe an (Untersuchungen noch nicht abgeschlossen).

- 3.3 Die Exposition dieser Arten kann als Richtlinie ausgearbeitet werden. Sie erlaubt eine rasche "Einleiterqualitätsbestimmung" im Sinne des "Abwasserabgabengesetzes" (Bundesrepublik Deutschland) und des "Abkommens über den Schadenersatz bei grenzüberschreitenden Umweltschäden".

Das Expositionsverfahren kann, unter Beachtung von Vitalität und Schadstoffaufnahme der exponierten Organismen und bei gleichen hydrologischen Bedingungen der Vergleichsstandorte, als Bewertungssystem für die relative Toxizität eines Gewässers oder eines Einleiters angesehen werden. Bedenkt man, daß keineswegs alle chemisch belastenden Stoffe apparativ nachgewiesen und überwacht werden können, so ist der "Wirkungskataster" das billigste und beste Instrument für die Bewertung von Belastungen. Da *Physa acuta* und *Dreissena polymorpha* in allen Gewässern der EG entweder vorkommen oder "eingesetzt" werden können, ist ihnen u.E. der Vorzug zu geben.

Zusammenhänge zwischen der Saarb Belastung (Einleiterzahl, Sauerstoff- und Temperaturverlauf im Juni/Juli 1975, BSB- und CSB-Wert, NH_3 , NO_2 - und SO_4 -Konzentrationen im Juni/Juli 1975), der Artenzahl und Diversität (H' -Wert) von Molluskenpopulationen sowie dem Überlebensindex in Boxen exponierter Organismen. Während im Fließgewässer vorkommende benthale Organismen ihre Verbreitungsgrenze bei der Rossel-Einleitungsstelle erreichen, überleben 20 bis 40 Prozent in Boxen exponierter Organismen in den rhithralen Flußabschnitten.



4. Publikationen im Rahmen des Projektes Nr. 012-74-IENVD

- MÜLLER, P. (1975): Ökologische Kriterien für die Raum- und Stadtplanung. Umwelt-Saar 1974 : 6-51.
- MÜLLER, P. (1976): Voraussetzungen für die Integration faunistischer Daten in die Landesplanung der Bundesrepublik Deutschland. Schriftenr. Vegetationskunde 10:27-47, Bonn.
- MÜLLER, P. (1977): Biogeographie und Raumbewertung. Wiss.Buchges. Darmstadt.
- MÜLLER, P. (1977): Belastbarkeit von Ökosystemen. ENVITEC, Düsseldorf.
- MÜLLER, P. und SCHÄFER, A. (1976): Diversitätsuntersuchungen und Expositionstests in der mittleren Saar. Forum Umwelthygiene 2:43-46.
- SCHÄFER, A. (1976): Zur Frage der Einwanderung von *Potamopyrgus jenkinsi* (SMITH 1889), *Physa acuta* DRAPARNAUD 1805 und *Dreissena polymorpha* (PALLAS 1771) in die Saar. Faun.-flor. Notizen aus dem Saarland 1:9-13.
- SCHÄFER, A. (1976): Diversitätsanalysen von Molluskenpopulationen und Expositionstests als Kriterien für die Darstellung ökologischer Probleme der Saarkanalisierung. Faun.-flor. Notizen aus dem Saarland 8 (3-4): 1-16.
- SCHÄFER, A. (1977): Möglichkeiten und Bedeutung quantitativer Erfassungen von Benthoszönosen in einem anthropogen überformten Fließgewässer, dargestellt am Beispiel der Saar. Ber.Internat.Symp. Vegetationskunde. Verl. Cramer, Vaduz.
- SCHÄFER, A. und MÜLLER, P. (1976): Auswirkungen der Saarbelastung auf die Speziesdiversität von Benthosbiozönosen und die Verweildauer exponierter Organismen. Verhdl. Ges. Ökologie, Wien.

Contractor: Universität des Saarlandes
D 6600 Saarbrücken

Contract n^o: 065-74-1 ENVD

Project leader: Professor Dr. H. Kaltwasser

Title of project: Accumulation and removal of inorganic
poisonous substances and trace elements
by microorganisms.

1. Concentration and distribution of toxic trace metals were studied in the river Saar and its affluents using atomic absorption spectrometry (AAS). The elements Fe, Mn, Zn and Pb were determined in the flame. For Co, Ni, Cu, Cd, Cr, V and Mo a graphite furnace was employed; Hg was measured using a newly developed device which allowed to determine 0,03 µg/l Hg in aqueous solutions. Biological material was treated with nitric acid under pressure prior to AAS analysis.

The results obtained from various sampling stations along the entire river, revealed drastic fluctuations in trace element content. Maximum concentrations were observed in the area of Völklingen/Dillingen (obviously due to the tributaries Rossel and Prims) and near Merzig/Mettlach, where ceramic factories are located. Lower concentrations were observed in between and after these areas, obviously due to sedimentation. At pH 8, the elements Pb, Fe, Cr, Cu, Zn and Cd were predominately bound to the particulate fraction, while most of the V, Ni, Mn, Co and Mo was in solution. Extreme quotients of distribution between particles and solution were observed in case of Pb (13:1) and Mn (1:5). Also, the sediments showed considerable local fluctuations with regard to their metal content. Highest values were observed in the case of iron (0,35 % Fe at Luisenthal), lead (1,9 % Pb at Mettlach) and Zinc (0,5% at Fremersdorf). The enrichment

factors between sediment and free water reached values between 10^3 for Mo and 4×10^5 for Pb. For these reasons the effect of heavy metals present in the particulate fraction needs to be considered during microbiological studies of this ecosystem.

2. With pure cultures of microorganisms belonging to different metabolic types (*Escherichia coli* K 12, *Alcaligenes eutrophus* H 16 and *Bacillus subtilis*), the effect of heavy metals on cell growth and respiration was studied. Half-maximum inhibition was determined and compared using 15 different metals at various concentrations. In manometric experiments, the influence of heavy metals upon bacterial respiration was determined.

Hg, Cd, Ni, Cu and Ag turned out to be most toxic against all tested strains. These strains, however, revealed different degrees of sensitivity towards certain metals, particularly Hg, Zn, or Cd. In case of *E. coli*, a 10^4 -times higher Cd-concentration was necessary to obtain a 50 % inhibition of growth and respiration as compared to *B. subtilis*. *B. subtilis* also turned out to be much more sensitive against Zn and Hg when compared with *E. coli* and *A. eutrophus*. In all strains tested, growth was inhibited by much lower concentrations of Sn, Cr and Be than respiration was. Likewise, growth and respiration were differently effected by Cu in case of *A. eutrophus*, and by V in case of *E. coli*. Moreover, the toxicity of heavy metals varied with most of these organisms depending on cell density and substrate concentration.

3. The numbers of nitrate and sulfate reducing microorganisms, and colony forming units (CFU) of aerobic, saprophytic and fluorescent bacteria were used to characterize water samples from different stations at the river Saar. Furthermore, the main chemical and physical parameters were determined.

Based on these results, the river Saar may be divided into three distinct zones:

- a) The upper part with generally low concentrations of all tested microorganisms. Temporarily, saprophytic microorganisms reached 10^5 CFU/ml, resulting from municipal sewage introduction, but bacterial concentration diminished quickly downstream.
- b) The middle region was characterized by the accumulation of municipal, industrial and thermal loading. Aerobic, saprophytic microorganisms reached up to 2×10^6 CFU/ml. These events caused severe oxygen depletion. The resulting impact on this ecosystem caused severe changes in the microbial nitrogenous metabolism also.
- c) The lower part, in which a partial recovery was observed. This, however, does not allow full mineralisation of the large amounts of organics or complete nitrification of the nitrogenous compounds present.

Using ^{14}C -glucose uptake kinetics in water samples, the effect of different heavy metal compounds upon the mineralization process was investigated. Several metal ions turned out to be inhibitory when added to water samples in ppb concentrations, showing the following range of toxicity: Hg, Cu, Pb, Zn, Cd, Sn, Ni, Co, Fe.

According to these results, the actual concentrations of heavy metals observed, might well be able to inhibit microbial activities such as mineralization in the river Saar.

4. Protozoa, able to grow with *A. eutrophus* H 16 as sole nutrient source, were enriched from water and soil samples. Axenic cul-

tures were obtained by isolation procedures using a micromanipulator. Seven species of protozoa showed to be able to grow in mixed cultures with *A. eutrophus* H 16. Growth curves were monitored in several experiments, and procedures were developed to separate protozoa and bacteria, using filtration and centrifugation techniques.

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- Blaß, M.: Mikrobiologische, chemische und physikalische Untersuchungen der Saar im Längsverlauf und in Abhängigkeit vom Jahresrhythmus. Diplomarbeit, Universität Saarbrücken, 1977.
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Contractor: Institut für Ingenieurbiologie und Biotechnologie
des Abwassers, Universität Karlsruhe

Contract n^o: 1o2 - 75 - 1 ENVD

Project Leader: Prof.Dr.rer.nat. L.Hartmann

Title of project: Einfluß von Metallgiften auf Flußbiocoenosen

The research was designed to evaluate the impact of heavy metal ions on microbial biocoenoses. One of the most problematic effects caused by toxic water pollutants consists in the inhibition of biological self-purification, i.e. the inhibition of bacterial metabolic activity. Bacterial metabolism is to be understood as a complicated network of reaction sequences, the smallest unit being an enzymecatalyzed reaction. Under the assumption of one rate limiting step the Michealis-Menten equation for enzymecatalyzed reactions

$$v = V_{\max} \frac{S}{K_m + S}$$

may be applied to substrate elimination by bacterial cells. As shown by Hartmann (1), Wilderer (2) and Singrün (3), even the substrate elimination by mixed cultures such as activated sludge exhibits Michaelis-Menten kinetics. In this case the overall reaction represents the summation of all single reactions taking place in the system. The results obtained, therefore, do not allow one to draw conclusions about the nature of the rate limiting enzyme or the organisms involved. Nevertheless, they enable a mathematical description for the case of the mixed culture in question, which may be useful for all practical purposes.

The fact that the self-purification activity of mixed cultures may be described by Michaelis-Menten kinetics suggests the possibility of describing and interpreting the toxicity of heavy metal ions towards river biocoenoses by using the standard formulations for enzyme inhibition.

There are several different types of enzyme inhibition, the three main types being competitive, noncompetitive and uncompetitive inhibition. In competitive inhibition the inhibitor affects the binding of substrate, which results in increased K_{mI} -values; in noncompetitive inhibition it affects the conversion into product, which results in decreased V_{maxI} -values. If the inhibition is uncompetitive both functions of the enzyme are affected and both parameters - K_{mI} and V_{maxI} - decrease. This type of inhibition, however, occurs very seldom.

The investigations were carried out using several different biocoenoses from a laboratory scale model river as biological test material and Na-acetate as substrate. No buffer was used in order to avoid precipitation of heavy metal ions. Four heavy metals were tested: Hg, Zn, Cu and Pb.

All investigations of heavy metal toxicity by means of the formulations for enzyme inhibition were conducted using the Warburg technique. In aerobic metabolism the conversion of substrate requires an equivalent consumption of oxygen. Therefore, substrate conversion may be measured with respirometric methods. In the Warburg technique, oxygen consumption is measured as a decrease in pressure in a closed system.

Batch experiments were carried out to evaluate the immobilization (precipitation, adsorption, cation exchange) of Hg^{2+} -ions in the model ecosystem and to observe the regeneration of metabolic activity after a slug dose of 6 mgHg/l.

Concerning aquatic ecology, methyl mercury, which accumulates in aquatic organisms, is the most important form of mercury. It is known that microorganisms are able to synthesize methyl mercury from inorganic mercury. Therefore some batch experiments were carried out to investigate whether the biocoenoses from the model river were capable of Hg-biomethylation.

The investigations yielded the following results:

- 1) The inhibitory effect of heavy metal ions on the metabolic activity of microbial biocoenoses may be described as a noncompetitive inhibition; V_{maxI} decreases with increasing heavy metal concentrations.

- 2) Due to a change of the rate limiting step, the inhibition of living cells may exhibit deviations from the kinetics valid for the inhibition of purified enzymes. The superimposing of several different reactions in overall mixed culture metabolism may also cause deviations.
- 3) Although the heavy metals examined cause the same type of inhibition, the special characteristics of inhibition are different for each metal.
- 4) In Hg-inhibition the maximal velocity ($V_{\text{max}}^*_{\text{Hg}}$) depends on Hg^{2+} -concentration according to

$$V_{\text{max}}^*_{\text{Hg}} - V_{\text{max}}^* = \frac{\text{Hg}}{a + \text{Hg}} \quad b$$

in which a is the Hg-concentration which yields $b/2$ and b is the maximal inhibition. Hg-inhibition is not linear, i.e. it cannot be linearized in the representation of Dixon, plotting $1/V_{\text{max}}^*_{\text{Hg}}$ versus I . This deviation from linear noncompetitive inhibition is probably due to a change in the rate limiting step.

- 5) Zn-inhibition is a partial noncompetitive inhibition and is therefore also nonlinear. For $\text{Zn} \rightarrow \infty$ a remainder of metabolic activity persists. The inhibition may be written as

$$V_{\text{max}}^*_{\text{Zn}} = V_{\text{max}} \frac{K_i}{K_i + \text{Zn}} \quad ;$$

this formulation, however, is valid only for small Zn-concentrations.

- 6) In Cu-inhibition two Cu^{2+} -ions are bound to the active centre of one enzyme. The maximal velocity therefore decreases according to the equation

$$V_{\text{max}}^*_{\text{Cu}} = V_{\text{max}}^* \frac{K_i}{K_i + \text{Cu}^2}$$

- 7) Pb is the least toxic of all heavy metals investigated. The dependency of $V_{\text{max}}^*_{\text{Pb}}$ on Pb concentration may be written as

$$V_{\text{max}_{\text{Pb}}}^* = V_{\text{max}}^* \frac{K_i}{K_i + P_b}$$

Due to a change of the rate limiting step, this expression is valid only for a limited range of Pb-concentrations.

- 8) Biocoenoses containing a high share of active organisms and consequently exhibiting high V_{max}^* -values are most susceptible to inhibition by heavy metal ions.
- 9) Hg^{2+} -ions added as a slug dose are eliminated from the solution in a very short time (ca. 10 minutes). This immobilization, however, is not to be understood as an inactivation. The inhibition of the biocoenoses concerned lasts for days.
- 10) The biocoenoses of the model river are capable of synthesizing organic mercury compounds from inorganic mercury.

Definition of symbols

I:	inhibitor
K_i :	inhibitor constant
K_m :	Michaelis constant
K_{mI} :	Michaelis constant in presence of an inhibitor
S:	substrate
v:	reaction velocity
V_{max} :	maximal reaction velocity
V_{max}^* :	maximal reaction velocity related to biomass concentration
V_{max_I} :	maximal reaction velocity in presence of an inhibitor

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Engelmann G., Wilderer P., Hartmann L.: "Modellfließgewässer zur Analyse der ökologischen Wirkung von Umweltchemikalien" WAF (in Vorbereitung)

Contractor: Gesellschaft für Strahlen- und Umweltforschung
mbH München

Contract No.: 116-75-1 ENVD.

Project leader: Prof. Dr. F. Korte

Title of project: FATE AND EFFECTS OF PERSISTENT ORGANIC XENOBIOTICS
IN FRESH WATER

1. Results of Experiments with a Microecosystem

Experiments carried out with a laboratory simulation model ecosystem which will not be described here in detail have been discontinued. The results obtained from this model up to now are sufficient for comparisons with data of the outdoor experiment and for assessing the predictability from laboratory data to outdoor conditions. As test substances, the following three radiolabelled polychlorinated biphenyls (PCB's) were used: 2,2'-dichlorobiphenyl, 2,5,4'-trichlorobiphenyl, and 2,4,6,2',4'-penta-chlorobiphenyl. Additionally, for comparison, a possible PCB-substitute (chloroalkylene-9) was investigated as a ^{14}C -mixture. Surprisingly, the biomass residues of the PCB-substitute which is a mixture of 2,4'-dichloro-biphenyl and isopropylated derivatives were higher than those of the PCB's tested. On the other hand, the conversion rate of the substitute in the biomass as well as in the nutrient medium was higher than that of the PCB's. Furthermore, the substitute had a significantly higher toxicity for daphnia.

Further test substances were hexachlorobenzene, a chlorinated phenol and p-chloroaniline, the latter two in a simplified screening test. As expected, hexachlorobenzene showed the highest persistence, also when compared to the PCB's.

2. Outdoor Experiments

2.1 General

In April 1976, in a marshy meadow situated on a slight slope, six pits with $6 \times 8 \text{ m}^3$ volume were excavated. These were filled immediately with ground water and formed ponds in which, during the summer months, a natural

biomass developed until an ecological climax was reached. The experiments with environmental chemicals were begun in August 1976.

2.2 Pentachloronitrobenzene- ^{14}C (PCNB)

The substance was applied in pond number 5, the pond number 2 situated somewhat higher served as a control. The first application was carried out on August 1 and was followed by seven further applications at regular intervals up to October 3. During this time, it was tried to adjust the chemical concentration to a constant value of 0.045 mg/l (expressed as radioactivity of the parent compound). During the experiment, the mean concentration decrease in water was 42% within 48 hours, the deviations being 50 - 34% depending on weather conditions.

2.2.1 Sampling

On November 1, samples of sludge (upper sediment layer) were taken as well as the following plants: *Lemna triscula*, *Ranunculus aquatilis*, *Juncus acutiflorus*, *Juncus stygius*, *Carex acutiformis*, *Carex vulpina*, *Equisetum variegatum*. The following fauna samples were taken: *Dytiscus marginalis*, *Notonecta glauca*, *Colymbetes fuscus*, *Daphnia*, mosquito larvae, dragon-fly larvae, and zooplankton.

2.2.2 Physical Data of the Biotope

pH:	7.3
Mean water temperature:	18 ^o C
Mean water volume in both biotopes:	5 - 7 m ³

2.2.3 Results

On November 1, the number of germs in the pond treated with PCNB was 5000/ml, in the control pond 5500/ml.

The concentration of radioactive substances (expressed as PCNB) in sludge was 1.27 mg/kg (wet weight); 7.7% = 0.092 mg/kg thereof was not extractable with methanol.

First analytical data of the radioactivity measurements of plants showed an accumulation factor up to 100. The highest values were found to be about 5 mg/kg in rushes. In the different parts of plants, like roots, stalks, and leaves, no differences in concentration could be detected.

The average concentrations of radioactivity in insects were 2 mg/kg.

2.3 Hexachlorobenzene- ^{14}C (HCB)

HCB was applied to pond number 4; the biotope number 1 which was situated

somewhat higher served as a control. The first treatment was carried out on August 1. Five applications were performed at regular intervals up to October 3. During the application time, it was tried to adjust the concentration of radioactivity (expressed as HCB) to a constant value of 0.050 mg/l. The decrease of concentration in water was fast; after 48 hours, an average of 30% of the applied radioactivity could be detected in water; after 96 hours, only 8 - 9% was present.

2.3.1 Sampling

Identical to 2.2.1

2.3.2 Physical Data of the Biotope

Identical to 2.2.2

2.3.3 Results

The number of germs in the HCB-treated biotope was 8000/ml, in the control 3500/ml. The total residues (expressed as HCB) in sludge were 0.85 mg/kg; 24% of these (corresponding to 0.16 mg/kg) were not extractable.

The highest residues found in plant samples were those in rushes (2.5 mg/kg). Most of the plant samples showed residues between 0.5 and 1.5 mg/kg.

The aquatic insects contained radioactive residues between 1 and 2 mg/kg, the zooplankton less than 1 mg/kg.

2.4 Methods for Quantitative Measurements

The radioactivity of water samples was determined directly in a liquid scintillation counter, and the results were confirmed by extraction of larger water volumes. The sludge-, flora- and fauna-samples were analyzed by automatic combustion and liquid scintillation counting of $^{14}\text{CO}_2$. The determination of conversion rates, which was carried out thus far for a few samples only, was performed by TLC.

2.5 Comparison PCNB - HCB

Surprisingly, the accumulation of radioactivity in biomass and sediment was lower for HCB than for PCNB, even when the shorter exposure time is considered. Furthermore, the higher portion of residues which are not extractable with methanol after HCB-application seems to be noteworthy, since this was not expected for a highly persistent compound.

Finally, it should be mentioned that these data obtained from the outdoor experiments are preliminary results only.

Contractant : Université Paris VII

N° du contrat : 044-74-1 ENV F

Chef du projet : Professeur PUISEUX-DAO, Université Paris VII et Professeur RAMADE, Université Paris XI, avec le concours des Professeurs DA LAGE (Université Paris V), DEVILLERS et FOURNIER (Université Paris VII) et LUTZ (Université de Clermont-Ferrand).

Titre du projet : L'évaluation des effets écologiques des polluants de l'eau : toxicologie et conséquences sur les principales catégories d'organismes constituant les réseaux trophiques.

THEME I

SENSIBILITE AUX PESTICIDES ET AUX DETERSIFS DES DIVERS MAILLONS DES CHAINES TROPHIQUES AQUATIQUES

Les travaux effectués sur ce sujet se rapportent à des Algues unicellulaires (*Amphidinium*, *Dunaliella*, *Euglena*), à divers Invertébrés aquatiques (larves d'Insectes : *Chaoborus* et éphémères du genre *Cloeon*, Crustacés amphipodes du genre *Gammarus*), à des têtards d'Amphibiens (*Bufo bufo*, *Xenopus laevis*), à des alevins de Truite (*Salmo irideus*).

EFFETS DES PESTICIDES

Les toxicités aiguës et à long terme ont été étudiées pour divers insecticides et un mélange d'herbicides (2, 4 D + 2, 4, 5 T).

La détermination des CL 50 dans les espèces animales étudiées après 24, 48 et 96 heures montre que la hiérarchie de toxicité aiguë reste à peu près constante entre les pesticides considérés, quelle que soit la position des organismes. Elles se rangent dans l'ordre décroissant suivant : fenthion > lindane > carbaryl > 2, 4 D + 2, 4, 5 T.

La CL 50 la plus faible est de l'ordre de la ppb avec le fenthion, la plus élevée de 15ppm pour les herbicides précités.

L'effet toxique le plus rapide s'observe avec le lindane chez *Gammarus*, le plus lent avec le carbaryl chez *Lymnea*.

La toxicité à long terme des produits se traduit par divers désordres physiotoxicologiques et démoécologiques. La croissance est inhibée par le lindane de manière variable chez les Algues planctoniques, (à partir de 0,3 ppm pour l'*Amphidinium*, de 5 ppm pour le *Dunaliella* l'Euglène n'y étant pas sensible) ; elle est ralentie chez tous les animaux exposés à ce produit. De surcroît s'observent des anomalies spécifiques : baisse de la fréquence des mues chez les Arthropodes aquatiques, absence de résorption de la vésicule vitelline chez les alevins de Truite.

Les études histopathologiques et en microscopie électronique ont révélé des altérations au niveau de divers organes : anomalies thyroïdiennes chez les têtards d'Amphibiens, lésion de l'hépatopancréas chez les Salmonides.

La fécondité est très généralement perturbée avec des doses infraléthales de pesticides. Si le mélange 2, 4 D + 2, 4, 5 T utilisé à 1 ppm ou moins a provoqué une augmentation transitoire de la taille des pontes de *Lymnea*, de telles concentrations sont, en règle générale, stérilisantes avec les insecticides testés.

Enfin, ce mélange d'herbicides et le lindane se sont avérés embryotoxiques tant chez les Invertébrés que chez les Vertébrés aquatiques étudiés à cet égard. Les principales anomalies concernent le déroulement de la blastulation et le développement du squelette cranio-axial.

EFFETS DES DETERSIFS

Sur les mêmes organismes ont été testés plusieurs déter-sifs : des anioniques (lauryl sulfate de Na, acide dodécyl benzène sulfonique linéaire, poudre de lavage du commerce comportant 80% de LAS et 20% de TBS), un non ionique (sel de sodium de sulfate d'alcools gras polyoxyéthylènes) et un cationique (bromure de lauryldiméthyl benzyl ammonium).

Fait paradoxal, c'est le déter-sif non ionique utilisé qui s'est avéré le moins toxique, les anioniques occupant une position intermédiaire entre ces derniers et le cationique.

Les larves d'Insectes testées sont assez résistantes aux détersifs, les CL 50 mesurées étant en règle générale supérieure à 10 ppm. A l'opposé, les *Gammarus* présentent une sensibilité maximale avec une CL 50 aussi faible que 0,37 ppm pour le cathionique étudié. Les *Lymnea* occupent une position intermédiaire et les écarts de CL 50 entre produits chimiques différents est faible, ce qui suggère que chez ces Gastéropodes, l'action tensioactive joue un rôle important sur le plan physiotoxicologique.

THEME II

RECHERCHES SUR LES POTENTIALITES STERILISANTES ET TERATOGENES DES PESTICIDES CHEZ LES VERTEBRES SUPERIEURS

OISEAUX

COMPOSES CHLORES

1. Anomalies de l'appareil génital

Après une courte immersion d'oeufs non incubés de Poule et de Caille dans une suspension aqueuse à 5% de DDT commercial dosé à 50% de substance active, on observe diverses anomalies au niveau des gonades et du tractus génital chez les embryons, les poussins et les adultes (embryons mâles : féminisation des testicules, réduction du nombre des tubules séminifères, maintien de vestiges müllériens; embryons femelles : altérations corticales et dilatation fréquente du canal de Müller droit).

L'étude expérimentale (greffes "*in ovo*" et cultures "*in vitro*") confirme les observations précédentes. Le DDT exerce un effet de type oestrogène.

2. Action sur la lignée germinale

Le pesticide induit une réduction hautement significative de la population germinale des gonades indifférenciées d'embryons de Poulet et de Caille. Ce déficit résulte de l'augmentation du taux de dégénérescence des cellules germinales et de l'inhibition partielle de leur pouvoir de colonisation des ébauches gonadiques.

3. Action sur le potentiel biotique

L'étude statistique des effets du DDT sur plusieurs

génération de Caille révèle que parallèlement à l'augmentation du taux de mortalité, le pesticide affaiblit fortement le pouvoir d'éclosion. Les Cailles femelles issues d'oeufs traités pondent moins d'oeufs que les témoins et plus tardivement. Le poids moyen des oeufs et des poussins est également réduit. Par contre, le DDT ne modifie pas le pourcentage de fécondation.

Des résultats similaires ont été obtenus pour un insecticide organochloré, le lindane (avec une baisse du taux de fécondation) et un herbicide, l'acide 2, 4, 5 trichlorophénoxyacétique.

COMPOSES PHOSPHORES

Des administrations intra-vitellines de parathion (>50 µg chez le Poulet et 10 µg chez la Caille) engendrent dans 100% des cas des troubles hautement spécifiques de la morphogénèse vertébrale (lordoses, cyphoses et scoliozes, associées à des soudures de vertèbres et à une désorganisation de la musculature vertébrale).

Alors qu'avec le parathion, les réponses tératologiques siègent spécifiquement au niveau axial, avec le dicrotophos (bidrin), elles affectent en plus le bec et les membres lorsque la dose employée est de 250 µg.

Les études embryologiques ont clairement montré que le tube neural et la corde (à rôles inducteurs et mécaniques) n'étaient pas impliqués dans les processus tératogéniques axiaux ; en effet des injections tardives de ces pesticides sont capables de produire des déformations cervicales même à des stades très avancés du développement (3ème tiers de la vie embryonnaire). Par contre, les anomalies axiales apparaissent lorsque débute la différenciation fonctionnelle du tissu contractile, en particulier de l'activité acétylcholinestérasique qui est totalement abolie par les traitements.

Les expériences de contre-traitements avec la nicotinamide et le tryptophane sont inefficaces pour prévenir les anomalies axiales (parathion et bidrin). Au contraire, l'amide nicotinique ou son précurseur biologique permettent une morphogénèse normale des structures appendiculaires et mandibulaires après action du bidrin. A l'inverse, la pralidoxime (P₂S, Spécia), ainsi que d'autres dérivés de l'aldoxime à noyau pyridinium (2-PAM et TMB₄), protecteurs et réactivateurs des cholinestérases phospho-

rylées, sont inefficaces pour contrecarrer les effets des deux pesticides sur les structures axiales, mais n'empêchent pas les signes tératogéniques spécifiques du bidrin sur le bec et les membres.

MAMMIFERES

CAPTANE

Le captane pur peu toxique par voie orale (DL 50 = 1,8 g/kg chez le Rat) l'est beaucoup plus par voie parentérale (DL50= 25 µg/kg chez le Rat et 22 µg/kg chez la Souris).

Chez les Rattes gestantes, le produit atteint les embryons (effets léthaux et fréquentes altérations du cristallin). Ces anomalies sont bien dûes au produit puisque des injections directes intra-amniotiques ou intrafoetales provoquent des cataractes bilatérales (à partir de 5 µg chez la Souris). On note alors aussi des troubles du développement des gonades mâles (longueur des tubes séminifères pouvant être réduite d'1/3 à partir de 10 µg).

Des expériences analogues d'intoxication aiguë sont également en cours avec l'acide 2, 4 dichlorophénoxyacétique.

THEME III

EFFETS DES PESTICIDES AU NIVEAU CELLULAIRE

LINDANE

Le lindane (pureté 95%) peu soluble dans l'eau a été dissous préalablement dans un solvant organique. Dans les conditions expérimentales choisies, les solvants seuls se sont montrés sans effet sur les cellules à l'exception d'une légère accélération du développement chez l'Acétabulaire avec le DMSO (0,2%). Dans l'ensemble des expériences, les effets du lindane ont été constants et particulièrement reproductibles.

Chez les Bactéries testées, le lindane n'a produit aucun effet sur la croissance des cultures. Chez les Eucaryotes végétaux, par contre, nous avons constaté une inhibition de la multiplication cellulaire à des concentrations variées suivant les espèces. Avec le *Dunaliella* (> 2 ppm) et l'*Amphidinium* (> 0,2 ppm), le déroulement de la division est perturbé et la cytotiérèse bloquée. C'est le cas également des cellules de

Végétaux supérieurs de racines d'*Allium*. Chez les Végétaux, le blocage de la cytotérièse est accompagné de différents phénomènes morphologiques (apparition de fragments membranaires erratiques observables en microscopie électronique dans les cellules de racines d'*Allium*, accroissement du volume cellulaire chez le *Dunaliella* et l'*Amphidinium*) et vraisemblablement de polyplôidisation.

Dans les cellules animales étudiées, hépatocytes en culture et chez le Protozaire *Tetrahymena pyriformis* G L, lorsque la croissance est ralentie ou inhibée, on n'observe pas d'anomalies mitotiques comme dans les cellules végétales. Ceci laisse supposer que le mode d'action de l'insecticide (ou de l'un de ses métabolites) n'est pas celui de la colchicine ou de la vinblastine ou bien qu'il y a plusieurs sites d'intervention de sensibilité différente pour les cellules animales et végétales ou encore que la pénétration du lindane n'est pas la même dans les deux types de matériel.

Le lindane ne semble pas agir non plus exactement comme la colchicine chez l'algue unicellulaire, *Acetabularia*, la colchicine inhibe seulement l'initiation du chapeau reproducteur mais pas sa croissance en diamètre alors que l'insecticide bloque les deux processus. Le lindane ne produit pas non plus des effets semblables à ceux de la cytochalasine qui, chez l'Acétabulaire, perturbe souvent brutalement les mouvements de cyclose avec comme résultat final une accumulation de masses cytoplasmiques dans les cellules ; au contraire, avec le lindane les mouvements intracellulaires s'arrêtent très progressivement et tout le siphon est rempli uniformément de cytoplasme.

Le noyau interphasique n'est probablement pas un des premiers organites-cibles. En effet nous n'avons pas observé d'anomalies ultrastructurales, excepté une augmentation de taille que l'on peut penser liée à des processus de polyplôidisation, tout au moins dans les cellules végétales. De plus, les autoradiographies réalisées avec l'*Allium*, après incorporation de thymidine - ^3H , montrent que même des doses provoquant un blocage complet des divisions permettent une synthèse du DNA nucléaire, tout au moins pendant 24 heures. Avec les hépatocytes, les capacités de synthèse de DNA persistent aussi ; elles sont cependant diminuées. Enfin l'inhibition des processus morphogénétiques par le lindane se réalise aussi bien avec des cellules nucléées qu'anucélées chez l'Acétabulaire.

Comme toute substance étrangère au métabolisme, le lindane provoque une hyperactivité golgienne et une augmentation du système vacuolaire. En outre le lindane est connu pour induire des systèmes enzymatiques "métabolisant les drogues". Cependant nous avons constaté que l'activité phosphatasique cellulaire était nettement plus faible que celle des témoins dans les cellules traitées, même pour les très fortes doses produisant une dégradation immédiate, au moins partielle des cultures.

Une hypothèse capable d'expliquer tous les résultats expérimentaux observés est envisageable : le lindane, très lipophile, intervient au niveau des membranes cellulaires ; ceci est suggéré par la résistance à des chocs osmotiques acquise par les Acétabulaires traitées. L'intégration de molécules d'insecticides dans la membrane plasmique, aboutissant à des modifications structurales pourrait être la cause de la baisse d'activité ATP asique $\text{Na}^+ - \text{K}^+$ signalée dans divers matériels traités par le lindane ; elle pourrait également perturber la formation des microtubules et microfilaments, soit en agissant sur leur ancrage, soit en modifiant les teneurs en Ca^{++} cellulaire.

Un tel mode d'action doit aboutir à une baisse diffuse du métabolisme chez tous les types cellulaires, pour lesquels n'existent pas de barrières à la pénétration de l'insecticide, avec blocage possible lorsque des activités nécessitent le fonctionnement de microtubules et microfilaments. Ceci permettrait de comprendre :

1°) pourquoi l'insecticide agit sur les Eucaryotes et a peu d'effet sur les Procaryotes (sans exclure un rôle des parois bactériennes sur la pénétration du lindane).

2°) pourquoi l'entrée en mitose se réalise avec des cellules végétales et pas les cellulés animales ; dans ces dernières, en effet, dès le début de la prophase, on assiste à des déformations cellulaires importantes avec formation et dédoublement d'asters alors que dans les cellules végétales, les mouvements actifs n'apparaissent qu'en fin de prophase.

Cette hypothèse pourrait expliquer les anomalies fonctionnelles du système nerveux des Insectes et celles observées accidentellement chez d'autres animaux dont l'Homme. Ces anomalies seraient alors dues à une concentration importante de lindane dans le tissu nerveux du fait de sa richesse en lipides et à une inhibition à la fois du transport intraaxonal et de la libération des vésicules au niveau des synapses. Il est bien évident

qu'une action au niveau de tous les systèmes sécréteurs est également à envisager.

D'après nos résultats, il est facilement concevable que des anomalies du développement aient été observées chez la Truite en présence de lindane, ce produit pouvant perturber aussi bien les mouvements des cellules embryonnaires que leurs divisions. Enfin, on conçoit, d'après les données décrites sur plusieurs Microorganismes que cet insecticide organochloré puisse provoquer des déséquilibres écologiques au niveau du plancton et en particulier faciliter le pullulement de Bactéries et probablement de Cyanophycées.

PICLORAME ET 2, 4 D

La méthodologie mise au point pour le lindane a été utilisée pour deux herbicides le piclorame et le 2, 4 D. Les résultats sont moins avancés, mais présentent des analogies avec ceux rapportés pour le lindane : inhibition de la morphogenèse cellulaire (*Acetabularia*) et de la multiplication des cellules, à des doses variables suivant les matériaux avec toujours une grande sensibilité des Dinoflagellés par rapport aux Volvocales.

De plus, des anomalies au niveau nucléaire ont été observées. En particulier, avec le piclorame pur aussi bien qu'en formulation technique, des corps nucléaires apparaissent ; ces structures avaient été décrites en pathologie humaine, principalement cancéreuse.

En outre, avec les deux formes de l'herbicide, on note des processus d'accumulation intracellulaire. Pour le piclorame, très nettement les impuretés accompagnant le produit technique peuvent augmenter considérablement la nocivité et favoriser des phénomènes d'autophagie cellulaire.

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 78350 Jouy-en-Josas, France

Contract N° : 045-74-1 ENV F, project 1

Project Head : R. BILLARD

Project Title : Effects of heat pollution and organo-chlorinated pesticides
 on fish reproduction.

I - EFFECTS OF TEMPERATURE ON TELEOST FISH REPRODUCTION

A - Effects on gametogenesis of goldfish (*Carassius auratus*) reproduction

Goldfish of both sexes were raised at 4 constant temperatures : 10, 17, 24 and 30°C, or submitted to two types of circadian heat fluctuations varying by 4°C around 17 and 24°C. A control put into natural environmental conditions was submitted to regular increase of temperature between February and August (10 to 27°C). The criteria used for evaluation were : plasmatic and pituitary levels of c-GTH gonadotropic hormone, evolution of R.G.S., quantitative analysis of gametogenesis.

The results show that at breeding temperatures equal to or higher than 17°C, with circadian variations or not, plasma c-GTH levels are always significantly ($P < 0.001$) higher than those of animals kept at 10°C for the first 4 months of treatment (February to May). In June and July, the situation is reversed and the fish raised in a high temperature present a lower level ($P < 0.05$) than subjects raised at 10°C. The pituitary c-GTH level in April is significantly higher at a high temperature ($> 17^\circ\text{C}$) than at 10°C ($P < 0.05$), while in June the reverse is seen, indicating a negative balance between synthesis and release at a high temperature.

Analysis of R.G.S. and gametogenesis shows that at a breeding temperature of 30°C, spermatogenesis and ovogenesis are very rapidly inhibited; in April only spermatogonia A are found. In groups raised at lower temperatures, most of the cell types are conserved and spermatogenic efficiency is maximum in April at 24°C and in June at 10°C. Such a study leads to very original conclusions : gonad and pituitary heat requirements are different. High breeding temperatures (30°C) inhibit gametogenesis by direct action on the gonads and enhance GTH secretion. It may be that this increase in gonadotropic secretion is due

to the suppression of a negative feedback usually exercised by the functional glands on the hypothalamo-pituitary system. This raises questions as to the physiological significance of these differential heat requirements, especially if it is remembered that the final heat preferendum of this species is about 30°C. It should be noted that this inhibition is reversible, and that resumption of gametogenesis has been observed after the fish have remained at 30°C for 6 months.

B - Effect on gamete survival and fertilization in the rainbow trout (*Salmo gairdneri*)

The experimental procedure was the following :

- effects on fertilization by submitting eggs and sperm to experimental temperatures only during insemination. Immediately after their collection, the gametes were progressively adapted over a 20-min period to experimental temperatures between 1 and 30 °C. Insemination was then practised with an insemination diluent (D.I.) previously brought to the same temperatures. The eggs were then left at room temperature for 10 min before transfer into fresh water incubators.
- effects on gamete survival. After dilution the gametes were independently exposed to experimental temperatures for 20 and 40 mins. Insemination was then practised using sperm and eggs left at 10°C. The effects were evaluated by the percentage of embryonic eggs observed after 10 days of incubation at 10°C.

The results show that the optimal fertilization temperature ranges between 5 and 15°C. At 20°C or more, fertilization rate diminishes, especially if dilution rate is high. When spermatozoa are put into movement after dilution in D.I., they retain their fertilizing ability longer at 0 and 5°C than at 10 and 15°C. If spermatozoa are immobilized by enriching the D.I. with K⁺, their fertilizing ability significantly decreases at 20°C and in some cases at 15°C. After 40 min of exposure, ovule fertilizability is only conserved at 1 and 5°C. It thus seems important to control the temperature during Salmonid insemination in fish-farming, and in natural conditions it is probable that temperatures higher than 15°C affect fertilization yield.

H - EFFECT OF LINDANE ON REPRODUCTION IN THE TROUT.

A.- Establishment of lethal doses

The objective of one of the proposed experiments was to administer non-lethal

amounts of lindane to trout during gametogenesis. These doses were determined in a preliminary experiment in which the dose of lindane was varied between 0 and 50 mg/kg of live weight per day. Mortality was found on day 3 of treatment for 50 and 25 mg doses and on day 9 for the 10 mg dose. Maximum mortality occurred on day 9 with the 50 mg dose and on day 20 with the 25 mg dose. After 3 weeks, mortality was negligible with the 10 mg dose; it was 20 % with the 25 mg dose and 50 % with that of 50 mg. The doses used in further experiments were 0.5 and 5 mg of lindane per kg of live weight per day, or 50 and 500 mg of lindane per kg of feed with a daily feed rate of 1 % of live weight.

B - Effect on gametogenesis

The breeding fish were fed during the period comprising the end of vitellogenesis and spermiogenesis (September, October) with feeds containing lindane (see II-A); the control was a commercial feed. Gametogenesis occurred normally, and all treated males and females spermiated and ovulated normally. The sperm fertilization rate of treated males did not differ from that of the controls. Although the ovules treated with the strongest lindane dose presented the highest relative lindane levels (3.3 ppm in relation to fat content), they presented a normal rate of fertilization and embryonic development comparable to the controls.

C - Effects on gamete survival and reproduction

The lindane being incorporated into the D.I., the experimental procedure is analogous to that described previously for testing the effects of temperature. Fertilization percentage decreased when the dose of lindane reached 25 ppm in the D.I. The effect was more marked when dilution rate was higher. It seems that fertilization rate was affected when the lindane was present in high amounts in the medium used for insemination.

III - EFFECTS OF SOME METALS ON GAMETE SURVIVAL AND ON FERTILIZATION. A PROPOSED TEST FOR TOXICITY.

With the methodology used in the experiments reported in paragraphs I-B and II-C, we carried out an experiment to test the toxicity of some metals introduced into the D.I. Hg, Fe, Cn, Cr are the most toxic for gametes.

It thus appeared that the methodology employed might constitute a toxicity test which could profitably complete already-existing tests and be used to evaluate the effects of various pollutions on a particularly sensitive period in the life of fish.

Contracting party : Institut National de la Recherche Agronomique
149 rue de Grenelle
75341 Paris Cedex 07 (France)

Contract number : 045-74-1 ENV - F, project 2

Head of the project : R. LESEL, Directeur du Laboratoire des Micro-organismes
B.P. 79, 64200 Biarritz (France)

Title of the project : Fish, pollution indicator : utilization of rainbow trout reactions to sub-lethal concentrations of toxic substances in water

General description of studies and results

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In the field of this contract, we tried to work out a detection test of the toxic effect of substances in solution in water at sub-lethal concentrations for a fish species : rainbow trout Salmo gairdneri. This test is simple, unexpensive and easy to carry out. Three apparatuses were designed :

- an electrical tester : research in the variation of the threshold of fish sensibility to electric current ;
- a rectilinear water current tester ;
- a circular water current tester in a funnel.

1. ELECTRICAL TESTER

1.1. Principle

The study was limited to the action of a detergent on fish sensibility to electric current. The hypothesis was that the threshold of fish sensibility to electric current is influenced by a chemical pollutant at sub-lethal concentrations. We tried to show up this fact by putting young trout for 24 h in solutions of various concentrations. The fish, placed in a trough between two electrodes, is submitted to increa-

sing electrical impulses, until it shows a reaction. Measures are made, the fish facing the cathode and the electrodes exactly fitting the fish length.

1.2. Working out

This apparatus was composed by an impulse generator, an electrode polarity reverser, a transparent plastic trough equipped with a fixed electrode and a mobile one giving the possibility to exactly adjust the trough useful length to that of the fish.

The toxicant used is an anionic detergent (Linear Alkylbenzene Sodium Sulfonate, activity 46.2% LAS), LC 50-24 h : 1.90 mg/l of active substance. Tests were made at 17° C.

The tested fish were rainbow trout kept in a rearing closed circuit.

1.3. Results and conclusions

With the four tested concentrations, it is noted that the averages of the threshold of sensibility are too close for their difference to be significant. The results obtained cannot therefore be utilized and the experimentation with that kind of apparatus was interrupted.

2. RECTILINEAR WATER CURRENT TESTER

2.1. Principle

This tester has been very much used to estimate the quality of fish intended for restocking. In our study, the fish introduced in a rectilinear pipe is submitted to a water current, the speed of which is increased by steps. The stamina of the tested fish is measured.

2.2. Working out

This apparatus was composed by a rectilinear glass pipe in which a constant water current is running (30 cm/s). This water current is created by a pump recovering, in a tank, the water coming out of the

pipe. A mechanical grid above the pipe prevents the swimming up of fish beyond the trial area ; an electric fence, down the pipe, stimulates the swimming of fish until exhaustion. Various systems (water gates, electronic control) enable to steady the water current inside the pipe.

The toxic substance used was the same as in the precedent case, the fish tested were coming from the same batch as in the electrical test.

2.3. Conclusions

Under a constant speed of 30 cm/s young trout offered resistance during many days. In these conditions, it was difficult to determine when the test was finished, without designing an apparatus much more sophisticated and therefore considerably more expensive. This apparatus was then eliminated.

3. CIRCULAR WATER CURRENT TESTER IN A FUNNEL

3.1. Principle

The fish is put inside a funnel in which the solution is propelled by a rotary motion round a vortex which caps the outlet. Thus the fish struggles against the water current (rheotactism) and against the vortex suction. When it reaches its stamina limits, the exhausted fish stops the funnel mouth ; the stamina duration of the fish is then measured.

3.2. Working out

3.2.1. Apparatus (fig. 1)

3.2.2. Tested substances

- an anionic detergent : Linear Alkylbenzene Sodium Sulfonate, activity 46.2% LAS, CL 50-24 h : 1.90 mg/l of active substance. The tests were made at 17° C ;

- a cationic detergent : Quaternary Ammonium Salt (Dimethyl Ditalow Ammonium Chloride), activity 75%, CL 50-24 h : 40 mg/l of

active substance. Tests were made at 17° C ;

- a non ionic detergent : Ethoxylated Fatty Alcohol, activity 99.9%. Tests were made at 20 and 24° C. CL 50-24 h at 20° C was 2.75 mg/l of active substance.

The swimming tests and CL 50 determinations were made with natural mineral waters of commercial distribution (Evian, Source Cachat) as reference water and dilution.

3.2.3. Tested fish : rainbow trout, length class 7/10 cm, kept in a rearing closed circuit.

3.3. Results (fig. 2)

It appears that the relation dose-effect is quite clear below a CL 50-24 h conventionnally established. The low concentrations of a toxic agent can be underlined by the test of fish swimming endurance. Tests show that it is possible to study in fish both effects of temperature and toxicity of substances in solution.

3.4. Conclusions. Future of the apparatus

The utilization of an active method leads to the increase of fish sensibility to the tested toxicants and allows to estimate the influence of low concentrations on it. A patent application for this apparatus has been presented. A further test for the toxicity estimation of an industrial rough effluent was carried out. It has been possible to show a qualitative alteration of water for fish, alteration which is a linear function of the toxic concentration. But the appreciation of a permanent pollution through a "fish-test" can be representative as far as the fish are all the time kept in the water to control and thus undergo all the fluctuations of environment characteristics. These observations led to the working out of a pollution biodetector, made on an industrial scale (Biodetector ERMAT) and which is the practical issue of the studies carried out within this contract.

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Fig. 1

Diagram of the circular water current tester

- 1 - Funnel
- 2 - Vortex
- 3 - Level indicator
- 4 - Buffer tank
- 5 - Electric clock
- 6 - Pump
- 7 - Control gate
- 8 - Electrical circuit

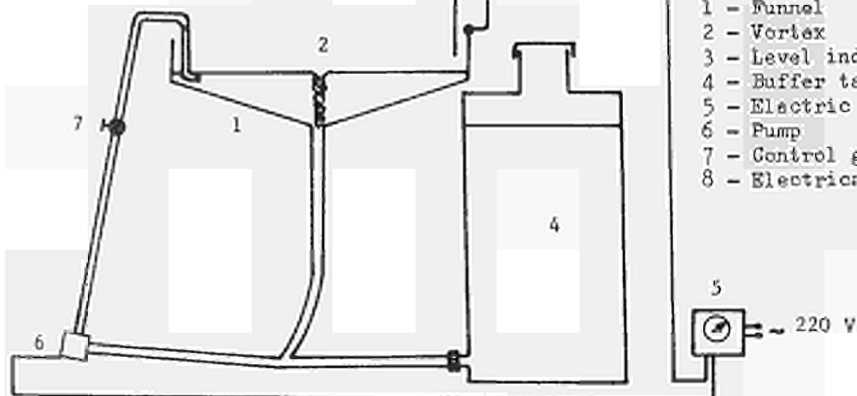
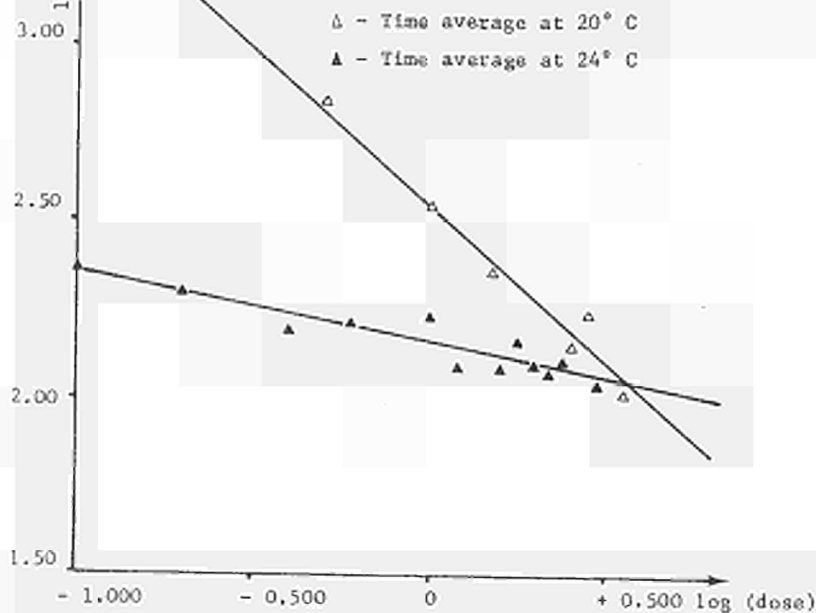


Fig. 2.- Relations between swimming resistance of young trout and non ionic detergent concentration at 20 and 24°C



Contractant : UNIVERSITE PAUL SABATIER TOULOUSE FRANCE

N° du contrat : O46-74 I ENVF

Chef du projet : Professeur A. SERFATY

Titre du projet : INTOXICATION PAR LE NITRATE DE PLOMB CHEZ LA CARPE.

Le plomb est libéré en quantité croissante dans l'environnement. Une partie est entraînée dans les rivières et risque d'être nocive pour la faune aquatique. Nous avons recherché les effets nocifs du plomb, à doses subléthale, sur l'organisme d'un Poisson d'eau douce, la Carpe (*Cyprinus carpio* L.), au cours d'une intoxication expérimentale prolongée suivie d'une période de récupération.

Les lots de Carpes (PI à P9) ont été intoxiqués pendant 1 à 9 semaines dans un bassin expérimental (volume : 400 litres, température : 20°C, débit : 120 L/h, concentration théorique en plomb : 10 g/l, plomb dissous: 3 à 4 g/l). Une partie des Carpes intoxiquées pendant 6 semaines ont été remises dans l'eau normale (sans Pb (NO₃)₂) pendant 1 à 5 semaines (lots DI à D5). Des exemplaires de chaque groupes ont été sacrifiés chaque semaine, pour suivre l'évolution de l'intoxication ou de la désintoxication.

Nous avons principalement étudié :

- la cinétique de fixation du plomb dans les organes et tissus par dosage au spectrophotomètre d'absorption atomique,
- la localisation du plomb dans les organes et tissus et les altérations morphologiques qu'il provoque,
- les variations de quelques paramètres métaboliques et physiologiques sous l'influence du plomb.

I. Cinétique de la fixation du plomb dans l'organisme.

La concentration du plomb augmente dans tous les tissus pendant l'irradiation, mais elle varie considérablement d'un organe à l'autre. Les dosages effectués nous ont permis de ranger ainsi les organes examinés par ordre de concentration croissante : muscles blancs et rouges, gonades, cerveau, coeur, sang, foie-pancréas, opercule, branchie, intestin antérieur, intestin moyen, contenu intestinal, rein abdominal et rate.

Nous avons plus particulièrement suivi l'intoxication et la désintoxication au niveau des organes suivants :

- a) La rate fixe une grande quantité de plomb (350 micro-grammes par gramme (ppm) de tissu frais en 6 semaines (P6), 680 ppm en 9 semaines (P9) d'intoxication). La désintoxication est très lente : 300 ppm à la dixième semaine (D10).
- b) Les reins abdominaux accumulent le plomb pendant l'intoxication (P9: 250 ppm) et le début du retour dans l'eau claire. Il s'élimine lentement.
- c) Le contenu intestinal contient du plomb bien que la nourriture soit normale. Pendant la désintoxication les teneurs élevées que l'on peut doser (D5 : 20 ppm) indiquent que l'intestin joue un rôle dans la désintoxication de l'organisme.
- d) L'intestin moyen fixe légèrement plus de plomb que l'intestin antérieur (P6 : 120 ppm contre 78 ppm). La désintoxication entraîne une chute rapide de la teneur en plomb (D10 : 7 ppm).
- e) Les branchies laissent pénétrer facilement le plomb dissous. On n'observe pas de précipité sur les lamelles branchiales. La teneur en plomb est élevée (P6 : 80 ppm) et l'élimination est lente (D10 : 23 ppm).
- f) Le foie et le pancréas, non dissociables chez la Carpe, accumulent moins de plomb que les organes précédents (P9 : 34 ppm). La désintoxication est rapide (D10 : 6 ppm).

II. Recherches histologiques de la localisation du plomb et des altérations morphologiques qu'il provoque.

Les réactions histochimiques montrent que le plomb est abondant dans le système réticulo-endothélial : cellules de Kuppfer, macrophages, et cellules lymphoïdes. Ces éléments sont très abondants dans la rate, le rein céphalique et le rein abdominal. Le plomb s'y accumule sous forme de grosses cellules riches en lysosomes très colorables par le chromate de Potassium.

Cinq organes ont été principalement prélevés :

- la branchie et le rein abdominal pour leur rôle dans l'élimination,
- la rate et le rein céphalique pour leur participation à l'hématopoïèse,
- le foie pour sa participation au métabolisme général.

a) La branchie n'est pas très rapidement altérée. On remarque, à la quatrième semaine, que l'épithélium respiratoire manque de cohésion et par endroit est détruit. L'inflammation se manifeste par l'afflux de mastocytes à la base et même à la surface des lamelles. La désintoxication

permet la régénération de l'épithélium mais non la disparition des mastocytes.

b) Le rein est légèrement congestionné. Les macrophages sont abondants dans les zones lymphoïdes entre les tubules.

c) Au niveau de la rate le saturnisme provoque de profondes altérations. En 2 semaines de nombreux macrophages se multiplient dans le système réticulo-endothélial et accumulent le toxique. On constate ensuite une importante diminution de la pulpe rouge, reflet de l'anémie saturnine. La mesure de de l'hématocrite confirme ces observations : voisin de 30 chez les témoins, il s'abaisse vers 18 chez les intoxiqués. Après désintoxication prolongée, l'hématopoïèse reprend mais les macrophages persistent. L'hématocrite s'élève lentement (D5 : 26).

d) Le foie et le pancréas sont tardivement atteints. Les macrophages ne sont abondants qu'à la 6^{ème} semaine d'intoxication. Le glycogène ne varie pas. On observe dans le foie et le pancréas ainsi que dans les tissus lymphoïdes des figures de dégénérescence (corpuscules de Hassal) en nombre important.

III. Recherche des modifications métaboliques accompagnant le saturnisme.

Les rapports organo-somatiques ne varient pas significativement que ce soit pour les reins, le coeur, les gonades ou le foie. Le rapport spléno-somatique diminue légèrement pendant l'intoxication.

Le métabolisme gluco-protéique a été étudié. Les teneurs en ARN total et protéines du foie, des muscles des reins et des branchies ont été évaluées par rapport au poids sec et par rapport à l'ADN. Le glycogène hépatique et musculaire ainsi que la glycémie ont été mesurés.

Dans le foie et les muscles les taux d'ARN et de protéines diminuent légèrement pendant l'intoxication et augmentent quelquefois au delà de ceux des témoins pendant la désintoxication. A la dixième semaine les taux sont normaux.

Le glycogène hépatique varie très peu, par contre le glycogène musculaire diminue fortement (40 %) pendant l'intoxication. Il se reconstitue lentement ensuite.

Dans les reins la concentration en ARN est supérieure à celle des témoins bien que la concentration en protéines ait tendance à baisser surtout au printemps. La restauration est assez rapide.

Dans les branchies la concentration en ARN augmente également

mais celle des protéines reste normale sauf au début de l'intoxication. La restauration est rapide.

En conclusion la Carpe peut accumuler des quantités importantes de plomb sans présenter aucun signe extérieur d'intoxication. Il est probable qu'il en est de même pour d'autres Poissons qui comme la Carpe, sont peu sensibles à l'anoxie. Ils peuvent être dangereux s'ils constituent un maillon important de la chaîne alimentaire à l'état frais, congelé ou sous forme de farine pour l'alimentation des animaux.

Publications.

- Intoxication par le nitrate de plomb chez la Carpe (*Cyprinus carpio* L) Influences sur le métabolisme respiratoire et la structure des filaments branchiaux. (Accepté pour publication au Journal Européen de Toxicologie).
- Intoxication par le nitrate de plomb chez la Carpe (*Cyprinus carpio* L.). Etude cinétique de la désintoxication. (en cours de rédaction).
- Intoxication par le nitrate de plomb chez la Carpe (*Cyprinus carpio* L.). Modifications métaboliques au cours de l'intoxication et de la désintoxication (en cours de rédaction).

Contractant : Commissariat à l'Energie Atomique - DPr - SERE -
 Section de Radioécologie - LPE - C.E.N. Cadarache -
 13115 - SAINT-PAUL-LEZ-DURANCE

N° du contrat : 047 - 74 - 1 ENV F - 1975 et 1976

Chef du projet : Monsieur GRAUBY A.

Titre du projet : Etude de la toxicité et de la fixation du zinc et du
 cadmium sur les anguilles et les daphnies.

I. TOXICITE DU CADMIUM ET DU ZINC SUR LES DAPHNIES

par

FOULQUIER L. avec la collaboration technique de JAULENT Y.

Nous déterminons les concentrations de cadmium et de zinc qui immobilisent 50 % des daphnies (*Daphnia magna* Straus) en 24 heures : CI.50. 24h. Nous recherchons l'influence du pH et de la dureté de l'eau sur ces toxicités. Enfin, nous étudions les effets synergiques ou antagonistes pouvant exister entre ces 2 éléments et le cuivre.

La méthode utilisée repose sur l'application de la norme expérimentale mise au point sous l'égide de l'Association Française de Normalisation : Norme expérimentale AFNOR T 90 301.

L'ensemble des résultats est indiqué dans le tableau ci-après.

- Le cadmium est environ 100 fois plus toxique que le zinc. La toxicité de cadmium n'est pas affectée par les variations de pH, tandis que celle du zinc l'est. Il a la même toxicité sous forme de nitrate ou de chlorure, ce qui n'est pas le cas du zinc. Aux concentrations utilisées, nous n'avons pas mis en évidence d'influence de la dureté de l'eau sur la toxicité du zinc vis-à-vis des daphnies.

- Le mélange cadmium-zinc est beaucoup plus toxique que le zinc seul mais beaucoup moins toxique que le cadmium seul. Le zinc a un effet inhibiteur pour le cadmium d'un facteur 10 environ.

- Le mélange zinc-cadmium-cuivre est nettement plus toxique que le zinc ou le cadmium seuls. Le sel de cuivre a un effet synergique sur la toxicité du zinc et de cadmium vis-à-vis des daphnies. Nous notons d'ailleurs que la CI.50.24h observée - 0,028 mg/l pour chacun des ions - est proche des concentrations maximales souhaitables indiquées dans la littérature : 0,005 mg/l pour le cadmium et 0,05 mg/l pour le cuivre.

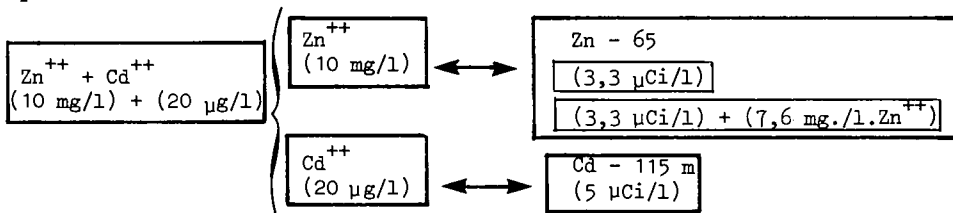
Composés utilisés	pH de la solution de départ	Expression de la CI - 50-24 h	CI-50-24 h corrigée (en mg/litre)
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$	4	Cd^{++}	0,12
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$	5,8	Cd^{++}	0,21
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$	7	Cd^{++}	0,18
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$	8	Cd^{++}	0,26
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$	9	Cd^{++}	0,22
$\text{Cd}(\text{NO}_3)_2, 4 \text{H}_2\text{O}$	5,1	Cd^{++}	0,15
Zn Cl_2	4	Zn^{++}	20,16
Zn Cl_2	6	Zn^{++}	25
Zn Cl_2	7	Zn^{++}	26
Zn Cl_2	8	Zn^{++}	1000
Zn Cl_2	9	Zn^{++}	$220 < \text{CI-50-24 h} < 280$
$\text{Zn Cl}_2 + 100 \text{ mg/l de Ca}^{++}$	6,3	Zn^{++}	21
$\text{Zn Cl}_2 + 1 \text{ g/l de Ca}^{++}$	6,3	Zn^{++}	23,9
$\text{Zn}(\text{NO}_3)_2$	4	Zn^{++}	5,28 et 37,4
$\text{Zn}(\text{NO}_3)_2$	7	Zn^{++}	40
$\text{Zn}(\text{NO}_3)_2$	9	Zn^{++}	$25 < \text{CI-50-24 h} < 40$ indétermination sur la valeur exacte
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$ + Zn Cl_2	6,2	Cd^{++} + Zn^{++}	1,2 + 1,2
$\text{CdCl}_2, 2 \frac{1}{2} \text{H}_2\text{O}$ + Zn Cl_2 + $\text{CuCl}_2, 2 \text{H}_2\text{O}$	6,2	Cd^{++} + Zn^{++} + Cu^{++}	0,028 + 0,028 + 0,028

Récapitulation des tests daphnies effectués sur le cadmium, le zinc et les mélanges cadmium - zinc et cadmium - zinc - cuivre.

II. ETUDE EXPERIMENTALE DE LA FIXATION DU CADMIUM STABLE, DU ZINC STABLE, DU MELANGE CADMIUM-ZINC, DU CADMIUM-115m ET DU ZINC-65 EN PRESENCE OU NON DE ZINC STABLE PAR ANGUILLA ANGUILLA (L)

par
PALLY M. et FOULQUIER L. avec la collaboration technique de LAMBRECHTS A.

L'ensemble des expériences de contamination à partir de l'eau peut se résumer ainsi :



Après différents essais, la méthode qui nous est apparue la plus fiable pour la détermination du cadmium et du zinc biologiques est la minéralisation nitrosulfurique suivie du dosage par spectrophotométrie d'absorption atomique en présence d'une lampe au deutérium. Dans certains cas, il faut effectuer une extraction à l'A.P.D.C. dans la méthyl-isobutyl-cétone.

- Après 76 jours dans une eau contenant 20 µg Cd⁺⁺/l et renouvelée tous les 15 jours, la teneur en cadmium de l'anguille est de 150 µg/kg frais. Les organes critiques de la contamination chronique de l'anguille sont les reins (2680 µg Cd/kg frais), les branchies (1990 µg Cd/kg frais), le foie (850 µg Cd/kg frais) et le tube digestif (720 µg Cd/kg frais). Nous avons globalement la même hiérarchie des valeurs avec le cadmium-115m (5 µCi/l).

Les facteurs de concentration du cadmium sont inférieurs à ceux déterminés pour les anguilles prélevées in situ ("témoins"), mais légèrement supérieurs à ceux obtenus après 30 jours avec le cadmium-115m.

La distribution du cadmium dans l'anguille "témoin" et celle dans l'anguille contaminée sont différentes. Nous observons une augmentation de la quantité de cadmium contenu dans les branchies et le tube digestif et une diminution de celle contenue dans le foie, les muscles et les reins.

Quelle que soit la durée de la contamination, nous avons à peu près la même distribution du cadmium stable dans l'anguille. Les organes critiques de la contamination représentent toujours au moins 70 % de la quantité totale de cadmium absorbé ; la peau, la tête et le squelette, environ 10 %. Cette répartition est relativement voisine de celle du cadmium-115m après 30 jours. Mais la distribution du cadmium-115m varie en fonction du temps. Le % de cadmium-115m retenu par les branchies diminue au cours de l'expérience (rôle dans l'absorption), par la suite le foie et les reins (rôle dans l'excrétion) en retiennent une part plus grande.

- Après 77 jours dans une eau contenant 10 mg/l et renouvelée tous les 15 jours, la teneur en zinc de l'anguille (68,4 µg/g frais) n'est que 1,4 fois celle des "témoins", alors que la teneur de l'eau est 500 fois celle d'une eau "non polluée".

La classification des organes par ordre décroissant de leur teneur en zinc, exprimée en $\mu\text{g/g}$ frais, est la suivante : les reins (258), la peau (132), le squelette (126), la tête (110,5), le foie (98), les branchies (92), le tube digestif (83), les muscles (57), le groupe rate-coeur-vessie nataoire (29) et le mucus (22).

Si nous comparons cette classification à celle établie lors de la contamination des anguilles à partir d'une eau contenant $3,3 \mu\text{Ci/l}$ de zinc-65, nous avons des différences notables : les branchies et les organes liés à la digestion et à l'excrétion ont les activités spécifiques les plus élevées.

Le zinc stable absorbé par l'anguille à partir de l'eau aux valeurs proches de la C.M.A. se distribue de la même manière que le zinc "naturel", tandis que la répartition du zinc-65 est différente.

Les anguilles contaminées par le zinc-65 et placées ensuite en eau inactive excrètent peu de zinc-65. Nous observons une redistribution de ce dernier dans l'organisme tendant vers celle du zinc "naturel".

Si la présence du zinc stable dans l'eau ($7,6 \text{ mg/l}$) modifie le transfert du zinc-65 de l'eau à l'anguille - il est 2 à 3 fois moins intense-, elle n'affecte nullement la distribution du zinc-65 dans l'anguille.

Toutes ces constatations nous permettent de dire que la fixation du zinc à partir de l'eau est bien régulée par l'anguille.

- Dans l'étude de la contamination chronique de l'anguille par un mélange de cadmium et de zinc sous forme de chlorures, nous voyons que la présence du zinc dans l'eau (10 mg/l) diminue la fixation du cadmium par l'anguille. Elle entraîne une diminution des teneurs en cadmium de la peau, de la tête, des branchies, du foie, des muscles et une augmentation de celle des reins et du tube digestif après 76 jours. Les organes critiques de la contamination sont toujours les reins, les branchies, le tube digestif et le foie.

La fixation du zinc par l'anguille n'est pas inhibée par la présence du cadmium dans l'eau à la concentration de $20 \mu\text{g/l}$.

Nous avons mis en évidence une compétition entre le cadmium et le zinc au niveau rénal ; dans le rein de l'anguille, à une faible teneur en cadmium et une forte teneur en zinc succèdent au cours du temps une forte teneur en cadmium et une faible teneur en zinc.

La distribution du cadmium absorbé à partir de l'eau par l'anguille est modifiée par la présence du zinc : les branchies ne sont plus l'organe principal de la contamination et la quantité de cadmium fixé par le tube digestif est beaucoup plus importante.

Par contre, il n'y a aucune modification de la répartition du zinc.

Contractor : Institut Européen d'Ecologie, Metz

Contract n° 049-14-1 ENV F

Project leader : J.M. Pelt

Title of project : Hydrobiological survey of the river "Moselle"

1. The objective of this report is to give an assessment of the water quality of a border-crossing river. The general evolution of different physico-chemical and biological parameters along the french course of the river (300 km) has been followed at thirty two sampling points throughout the four seasons of the year (1975).
2. The following remarks concern the methodology employed in assessing a large river like the Moselle :
 - . the sampling regime was chosen to enable a longitudinal section of the river to be assessed in terms of the variation in pollution load throughout the year.
 - . more information needs to be provided on some components of the ecosystem such as the phyto- and zooplankton and the benthos.
3. The river "Moselle" can be physico-chemically characterised as follows :
 - . there is an important mineral pollution at the beginning of the confluence with the "Meurthe" tributary causing water-hardness and a high conductivity.
 - . there is natural and artificially induced eutrophication.
 - . there are important differences in the water quality of the river's tributaries.
 - . an average physico-chemical index for twenty different parameters is proposed. This enables seven distinct sections of the river and some regeneration zones to be identified.
 - . a mathematical study shows a good correlation between the mineral flow and the longitudinal sections of the river. On the other hand, there is no relationship, except for nitrites, between the factors associated with the degradation of organic matter and the longitudinal sections.

This diagnosis reveals the environmental problem of the river. The self-purification processes are considerably modified by the variable industrial effluent input.

4. All the data obtained on the microbiology, algology, emerged macrophytes, zooplankton and benthic fauna, enable the biological structure of the river to be defined along its course.
- . The study of the pathogenic bacteria is of particular interest especially for total bacterial counts and coliforms. This allows the structure of the river to be divided into 6 sections with a general bent towards mesosaprobity. We have found good mathematical correlations between coliforms, faecal streptococci and longitudinal section.
 - . The planktonic algal community indicates a gradual eutrophication from upstream to downstream and permits the changes in the hydrobiological condition of the river in the whole to be followed. It is not possible to distinguish the pollution source with this method. However, some characteristic species of four sections have been identified.
 - . Emerged macrophytes also characterise four sections.
 - . For the planktonic fauna, Rotifera (Brachionidae especially) are good indicators and structure the "Moselle" into four sections. The barrage effect seems to have an important influence on the planktonic community.
 - . In the study of the benthic macrofauna, the use of a Biotic Index, together with a simple biocenotic analysis, discriminated five zones which are characterized by a specific faunistic association.
5. In this environmental survey, the complementary results of different methodologies, allow us to propose 8 typical sections of the river "Moselle". Each section can be distinguished by :
- its physico-chemical and biological structure
 - its degree of sensitivity to pollution
- We conclude that the river has a polluted zone followed by a regeneration zone.
6. These studies allow us to propose some species which are worthy of further study in greater detail as indicators of pollution :

. Microbiology : coliforms, total bacterial counts at 20° and 37° C, salmonellae and a faecal streptococci.

. Plankton - phytoplankton

Coscinodiscus lacustris (indicates salinity)

Cyclotella meneghiniana (indicates eutrophication and salinity)

- . Zooplankton

Rotifera brachionidae and Rotaria neptunia
(indicates organic pollution)

- . Filamentous algae :

Cladophora : (indicates eutrophication)

- . Macrophytes

Potamogeton pectinatus and P. crispus
(indicates increase in PO_4^{3-} and NH_4^+)

- . Benthos

Bryozoa

Snails : Bythinia tentaculata ; Radix sp
and Physa sp

- . Insects

Ephemeroptera, Trichoptera, Plecoptera in
zone 1, Chironomidae and odonata larvae,

- . Malacostraca : Asellus and Gammarus

- . Turbellaria, oligocheta and Hirudinea

- . Fish : Cyprinids population

Study of a simplified trophic chain of a freshwater ecosystem

The importance of an experimental model of a food chain has been demonstrated in the elaboration of new ecotoxicological tests.

The possibilities of diffusion, transformation and accumulation of toxicants may be studied in this kind of ecosystem model. We have defined the methodological principles and the necessary experimental conditions and the biological parameters which have to be considered. In this respect, we have studied a freshwater ecosystem constituted by 3 organisms belonging to the same mesological level.

1. Producers (*Chlorella vulgaris*)
2. Primary consumers (*Daphnia magna*)
3. Secondary consumers (*Leucaspius delineatus*)

These species have been studied separately according to bibliographical data. We have described the breeding and growing conditions used for, and tested the reproducibility of the biological parameters selected for each trophic level.

The growth of *Chlorella* has been monitored by three different methods :

- . the counting of individual cells with the "Thoma cell" under the microscope is precise but tedious and doesn't allow good experimental control.
- . the turbidimetric method enables growth curves to be established more rapidly and preserves the axenic conditions of the cultures. With regard to the recorded biological phenomenon, the reproductibility of this method seems satisfactory.
- . the determination of the chlorophyll concentrations with time. This criterion may be taken into account as some indication of the physiological condition of the culture.

As for the daphnids, we have selected the determination of duration of life and breeding capacities. Each criterion has been studied separately and the limits of its use and significance have been fixed.

Toxicological studies have been undertaken with several chemical substances. Among heavy metals, cadmium has been submitted to more extensive experimentation. Algae have been dosed at different cadmium concentrations to define growth inhibiting concentrations (GIC), such as GIC minimal, GIC₅₀, GIC₁₀₀ of cadmium metal (as a chloride). By that way, GIC₅₀ can be evaluated to 1 ppm for *Chlorella vulgaris*. The toxic levels of cadmium for daphnids and flies have to be defined according to time. The determination of the bioaccumulation levels will allow an estimate of the potential of the model in this respect to be made.

For this study, it was worthwhile to build a special and convenient apparatus with the maximum of automatization allowing a long term observation of the toxic events.

This methodology would seem to be useful in studies of the behaviour of ecosystem models in physiological pathology and toxicology.

Contractant : Université Paris 7
Groupe de Biotélemetry

N° du contrat : 055-74-ENVF 1

Chef du Projet : BERCY Claude

Titre du projet : " Application à l'étude du comportement des poissons à proximité d'une source chaude et à la détermination des zones de frai".

I - INTRODUCTION.

Les principales installations françaises de centrales thermiques ou nucléaires se situent en milieu fluvial ou estuarien. De nombreux travaux expérimentaux sont poursuivis par divers organismes afin d'étudier les conséquences de l'échauffement des eaux continentales sur la survie, et les fonctions physiologiques de poissons et d'invertébrés. Le laboratoire d'hydrobiologie de Montereau (EOF et CTGREF) a porté plus particulièrement ses efforts sur les conséquences écologiques de l'implantation des Centrales de l'Electricité de France, sur la vie piscicole. Les différentes techniques utilisées par les hydrobiologistes pour étudier les mouvements des populations piscicoles consistent principalement par : la capture des poissons, le marquage classique de ceux-ci, le repêchage et la sélection de ces derniers, des études statistiques. Aucune expérience en France n'a pu montrer précisément les déplacements précis et réels des poissons au voisinage d'une source chaude. C'est une des raisons principales de ce contrat par la mise en place d'un procédé automatique permettant de suivre les poissons par méthode ultra-sonore.

Nous rappellerons qu'une centrale est une énorme usine à réchauffer le milieu environnant, les eaux fluviales prélevées servant au refroidissement des condenseurs des turbines. Ces eaux aux contacts de ceux-ci s'élèvent de 6 à 10° Centigrade selon le type de centrale. La pollution thermique ne présente aucun degré de gravité en France, mais le développement énergétique français peut modifier considérablement l'équilibre thermique naturel. C'est dans le cadre de ces préoccupations que nos expériences se situent.

II-METHODE

La méthode utilisée consiste en la mesure de 3 distances que font le poisson porteur d'un émetteur-répondeur acoustique miniaturisé et trois hydrophones fixes, positionnés topographiquement. Ces distances sont calculés à partir du temps de parcours du son entre le transpondeur et les trois hydrophones des stations A, B, C. La vitesse du son dans l'eau étant V, les trois équations définissant les distances sont :

$$\begin{aligned} d_A &= V \times \frac{t_A}{2} \\ d_B &= V \times \left(t_B - \frac{t_A}{2} \right) \\ d_C &= V \times \left(t_C - \frac{t_A}{2} \right) \end{aligned}$$

d = en mètre
V = m/s
t = seconde

La figure n°1 présente le synoptique général de l'expérience :

- Dans la paroi abdominale du poisson est implanté le transpondeur acoustique (du Fisheries Laboratory de Lowestoft) ; ses principales caractéristiques sont : alimentation 4,5 V, fréquence d'interrogation 300 KHz, fréquence d'émission 93 KHz, sensibilité acoustique 50 μ B, niveau d'émission 68 dB/ μ B à -1m, dimension 74 x 14 mm , poids dans l'eau 19 gr.
- Une station A comprenant un ensemble d'interrogation et réception acoustique, un récepteur-émetteur de télémesure VHF (467,175, et 457, 175 MHz) un duplexeur d'antenne.
- Deux station B et C identiques comprenant un ensemble de réception acoustique un récepteur de télémesure VHF (l'un à 445, 450 , l'autre à 445, 350 MHz).
- Une station Centrale comprenant un ensemble électronique qui fixe les intervalles de mesure, effectue les chronométrages, calcule les distance et imprime les trois distances sur une imprimante. Elle a par ailleurs trois récepteurs de télémesure VHF réglés sur les fréquences d'émission des stations A,B, C et un émetteur VHF permettant de déclencher le système d'interrogation acoustique de la station A.
- un ensemble informatique comprenant :
 - . un calculateur programmable Hewlett Packard 9815 permettant , à partir des trois distances d'obtenir les coordonnées du poisson compatible .
 - . une interface BCD rendant compatibles les valeurs données par la station centrale et le calculateur.
 - . une table traçante en liaison directe avec le calculateur , les instructions de commande de celle-ci sont programmables dans le calculateur 9815.

La figure n°2 présente l'ensemble des télémesures radioélectriques VHF et acoustiques.

La figure n° 3 donne l'organigramme complexe de la station centrale. On remarque que l'on introduit dans la mesure de chronométrie des trois compteurs un certain retard correspondant aux différentes constantes de temps de l'électro-nique. Afin de pouvoir "rentrer" l'information vitesse du son dans l'ensemble de la localisation, une mesure de célérité du son est effectuée et affichée par roues condenses afin d'être pris en compte par l'ensemble du calcul. Il sera nécessaire de définir une température moyenne et être prudent sur la précision des mesures puisqu'une variation de 1°C correspond déjà à 3,5 m/s d'erreur.

III- RESULTATS.

Les résultats obtenus lors de différentes campagnes à la Centrale E.D.F. de Montereau ont montré la concordance entre les deux méthodes topographique et acoustique. La précision totale de la localisation acoustique vérifiée par méthode topographique donne une précision de l'ordre du mètre pour des distances de 500 mètres environ. Les caractéristiques du transpondeur utilisé imposant la limitation de cette portée.

Au cours de la campagne de janvier à mars 1977, quelques brochets ont pu être localisés pendant plusieurs jours sur table traçante (Figure n°4) dans le canal de rejet de la centrale de Montereau (Puissance 750 MW- $\Delta t = 6^\circ\text{C}$). Au vu de ces résultats on a pu définir que certains brochets séjournent dans des aires privilégiées, de nombreuses heures, en corrélation avec des frayères ou des accidents de fond dans le canal. Ces études de déplacements ayant été effectuées en période de frai. Une interprétation écologique de ces déplacements est en cours en liaison avec le laboratoire d'hydrobiologie de Montereau.

Actuellement, nos études de déplacements se situent à la sortie du rejet, afin d'étudier l'incidence des déplacements de poisson sur les isothermes des eaux de mélange.

Sur le plan biologique, une étude très complète (thèse de F. Zimmerman) a été entreprise sur l'effet de l'implantation d'émetteurs acoustiques sur le poisson. Il ressort de cette étude que les caractéristiques des émetteurs modifient quelque peu les différents facteurs étudiés : le poids des émetteurs provoque une diminution du nombre des interactions (sur une truite de 500 g, la baisse d'activité serait de 10% pour un poids de 5 grammes) ; la longueur des émetteurs ne modifie que l'activité spontanée ($\sqrt{10\%}$ pour une longueur de 3,5 cm) ; la survie atteint 75% , les 25 % de décès étant dûs à une mauvaise cicatrisation; l'opération abdominale ne perturbe pas le comportement des poissons.

Remerciements :

Nos travaux ont pu s'effectuer grâce à l'aide de Mr. MITSON du laboratoire de Lowestoft, à la Direction des Etudes et Recherches de l'EDF/ Département AAE, l'Agence Financière de Bassin Seine Normandie pour leur aide logistique et matériel, enfin le laboratoire d'Hydrobiologie de Montereau (CTGREF) pour toutes les questions piscicoles sur les lieux d'expérimentation.

IV - PUBLICATIONS

- ° BERCY C, VIGNERON J, GONDRAND B,
"Localisation automatique de poissons par triangulation acoustique" 2ème Congrès Européen des Ichthyologistes- UNESCO Paris Sept. 76
- ° BERCY C,
"An automatic system for localisation of fish-operation and results" - Symposium on Rhythmic Activity of fish - Stirling July 77
- ° ZIMMERMANN F, BERCY C,
"Etudes comparatives des méthodes de fixation d'émetteurs ultrasonores miniaturisés chez le poisson et puissance acoustique" 2ème Congrès Européen des Ichthyologistes - UNESCO Paris Sept. 76
- ° BERCY C,
"Application de la biotélémétrie aux recherches Ichthyologistes" présenté aux journées scientifiques d'Ecologie et d'Ichthyologie de l'EDF - Evry Octobre 76
- ° BERCY C,
"Expérience de localisation dynamique des poissons" journée d'Etude sur les conséquences éventuelles de l'échauffement des Eaux/Ministère de la qualité de la vie et Agence Financière de Bassin Seine-Normandie Juin 77
- ° THESES, DEA et rapports
 - F. ZIMMERMANN
"Effet de la fixation d'émetteurs chez le poisson- Utilisation de la méthode de tracking en Eco-éthologie.
Thèse de Doctorat de 3ème cycle soutenue le 7/07/77
 - B. GONDRAND
"positionnement d'un poisson par triangulation acoustique- Applications aux Centrales de l'EDF.
Thèse de Docteur Ingénieur à paraître 1er trimestre 78.
 - B. PIERRON
"Développements nouveaux dans le domaine des émetteurs acoustiques - Applications à l'Ichthyologie"
Thèse de Doctorat d'Université à paraître 1er trimestre 78
 - B. GONDRAND
"Positionnement d'un poisson par triangulation acoustique" rapport de DEA "Energie et pollution" Sept. 75
 - G. IPINO
"Emetteur radioélectrique miniature pour la retransmission de la température en milieu aquatique - Applications cartographiques et Ichthyologiques.
Rapport de DEA "Energie et pollution" Sept. 77
 - F. DUQUENNE
"Comparaison topographique et acoustique pour la localisation de poissons- méthode de calcul par trilatération et programme associé" rapport de fin d'Etudes Institut géographique national/Ecole des Sciences géographiques. Sept. 76
 - D. FAUCHER et F. VACCARO
"Réalisation d'un équipement électronique automatique pour le tracking de poissons au voisinage de Centrales nucléaires" rapport de fin d'Etudes ISEP Juin 75

LOCALISATION AUTOMATIQUE DE POISSONS
PAR TRIANGULATION ACOUSTIQUE

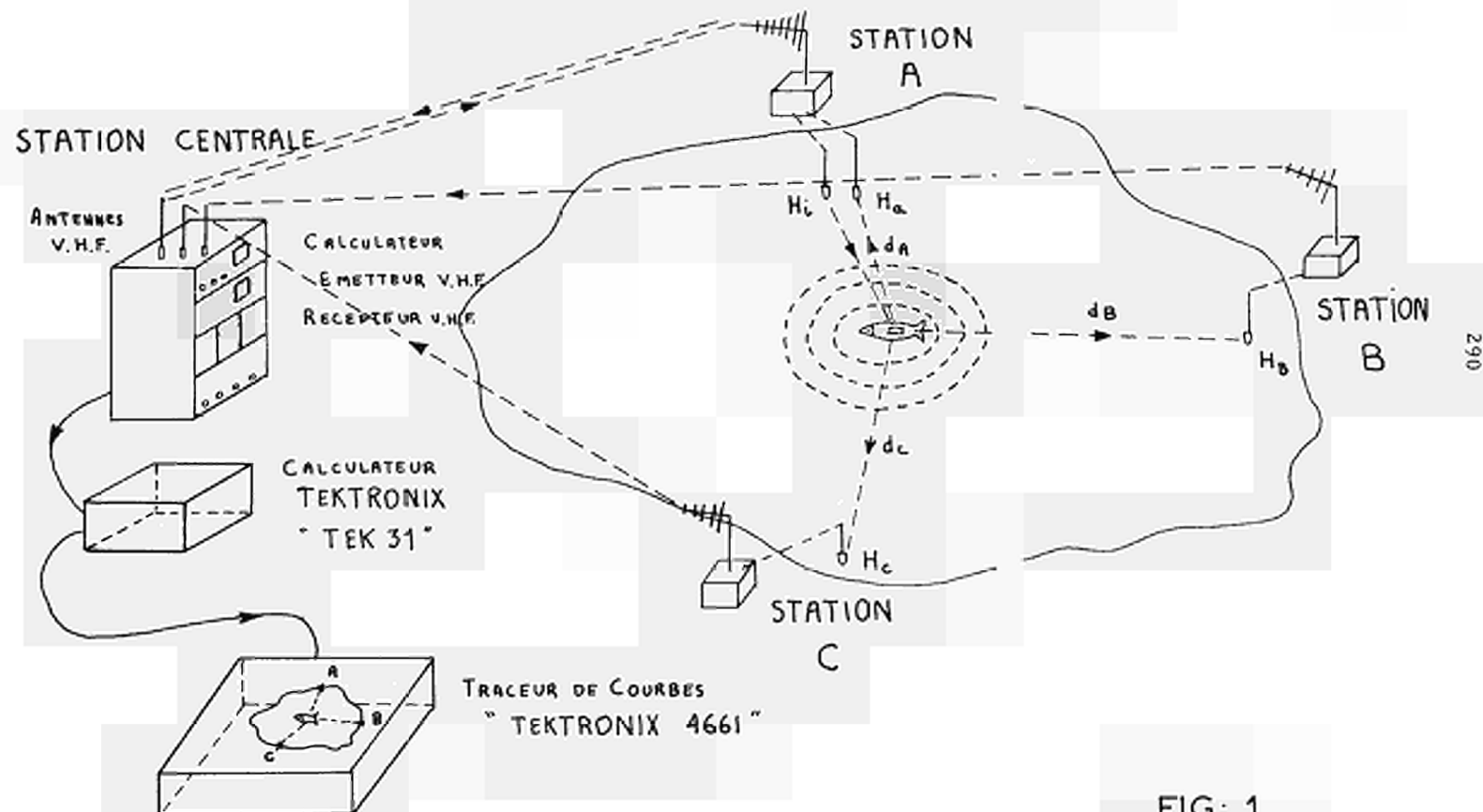
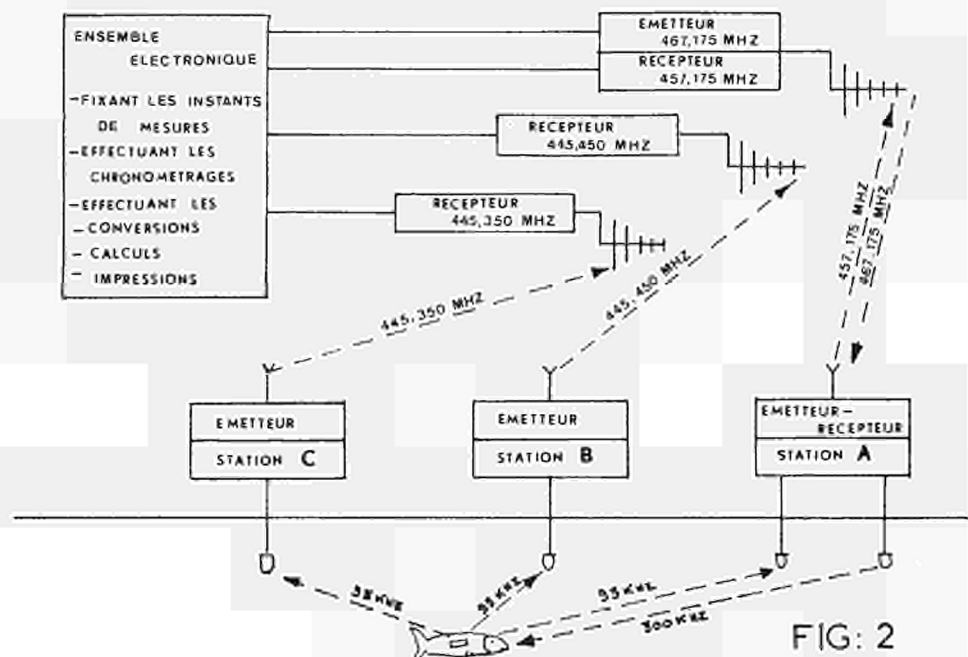
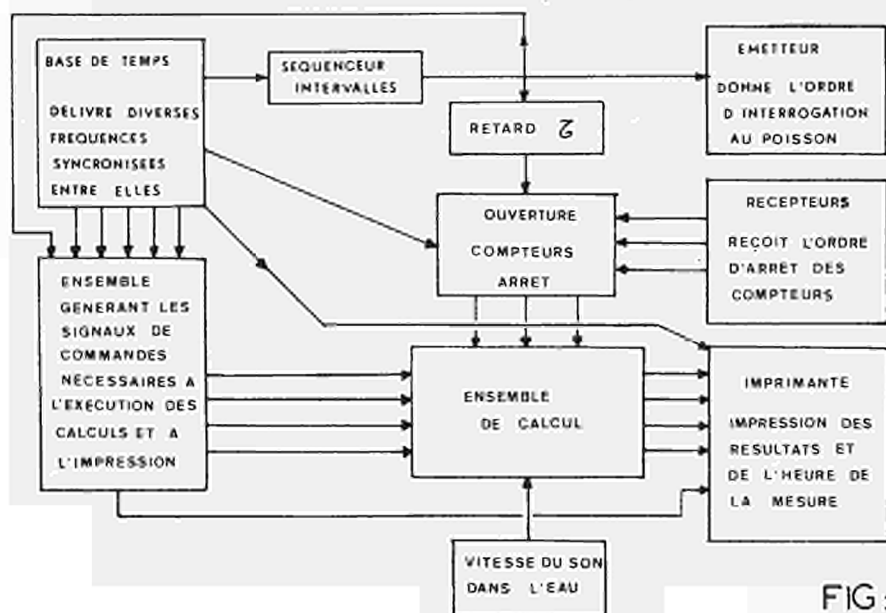


FIG: 1

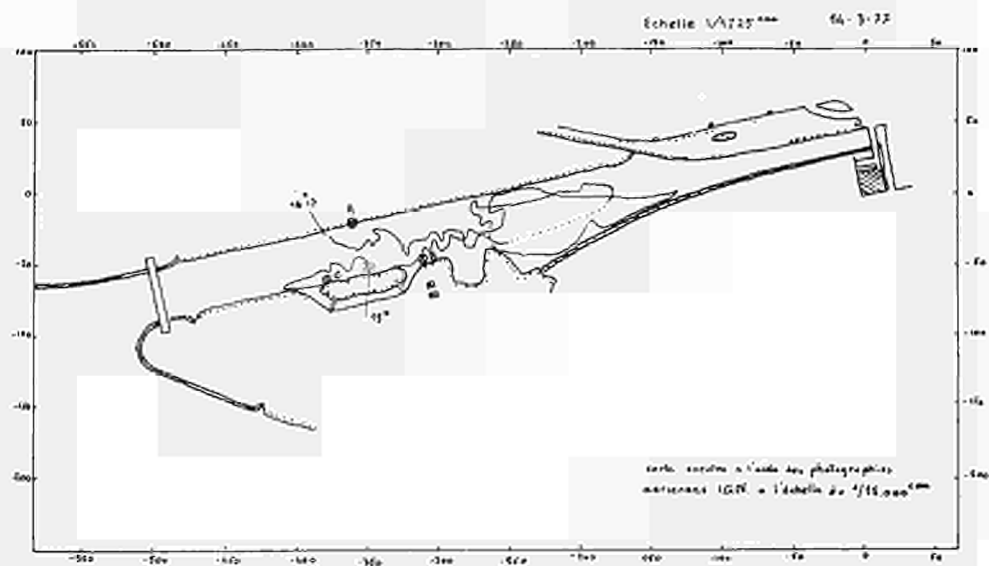
SYNOPTIQUE DES EQUIPEMENTS DE TELEMESURE



ORGANIGRAMME DE LA STATION CENTRALE



EXEMPLE DE TRACES DES DEPLACEMENTS DE POISSONS



CANAL DE REJET DE LA CENTRALE E.D.F. MONTEURAU

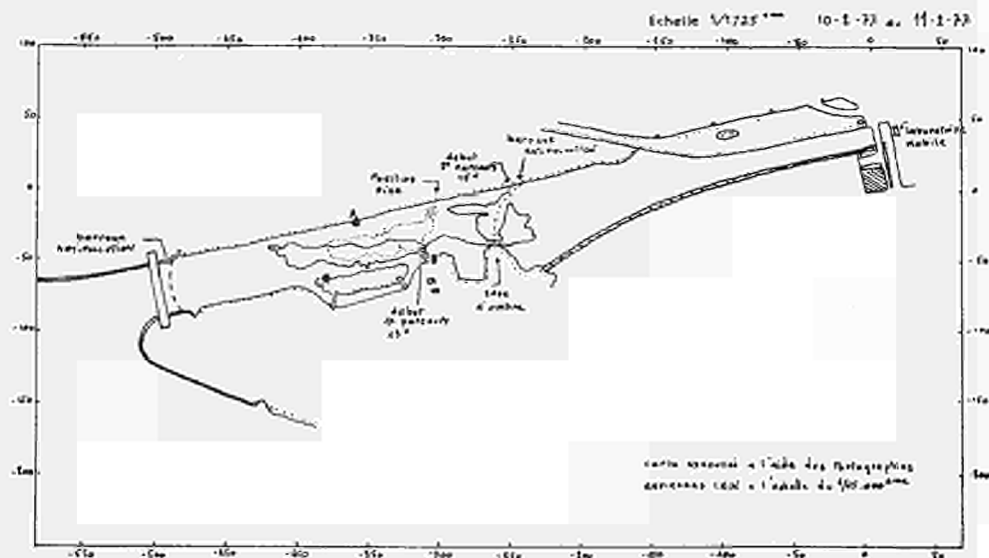


FIG:4

ORGANIGRAMME DU CALCUL DE TRILATERATION PAR ITERATIONS

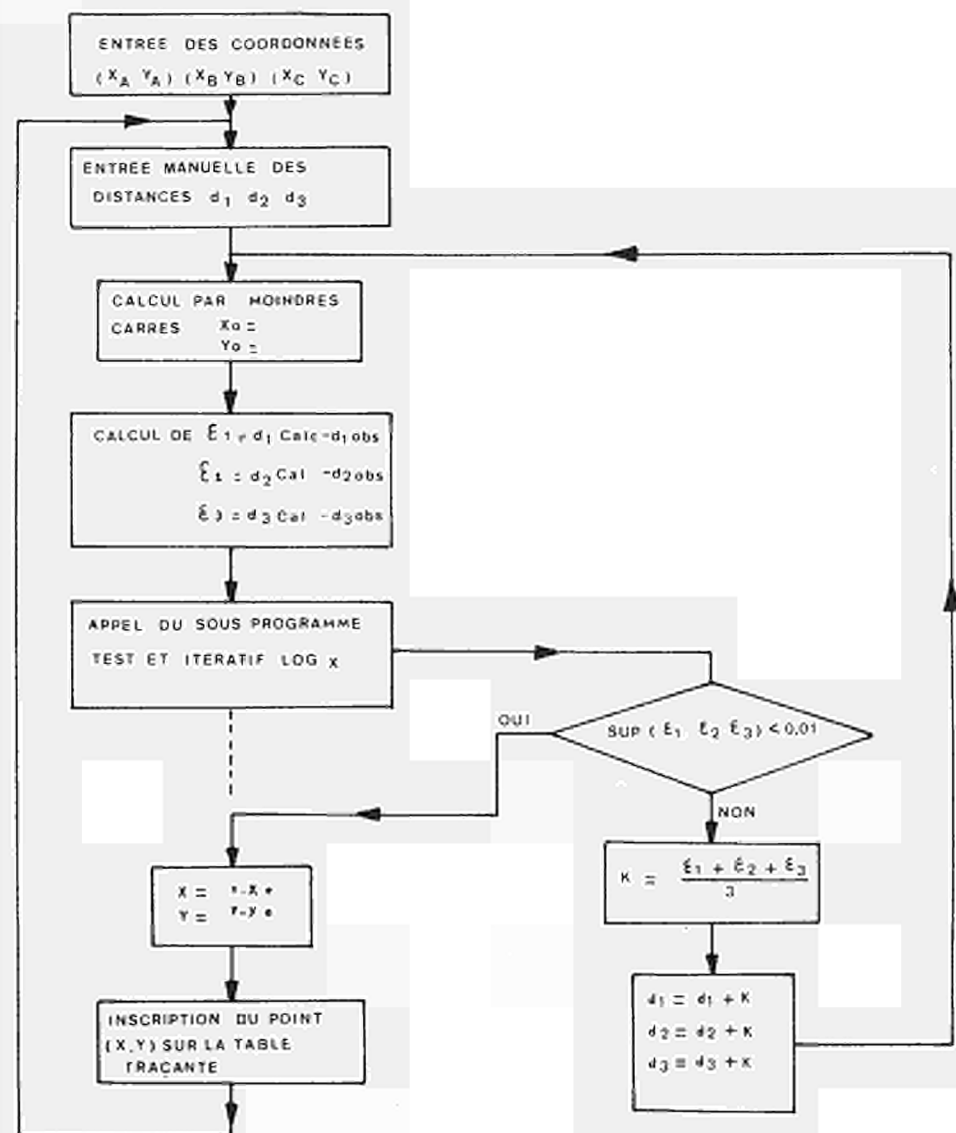


FIG: 5

Contractant : COMMISSARIAT A L'ENERGIE ATOMIQUE
C.E.N., Grenoble

N° du contrat : 100-75-1 ENV F

Chef du Projet : A. CORNU

Titre du projet : EFFETS ECOLOGIQUES DANS LES EAUX DE SURFACE DE
PRODUITS DE TRANSFORMATION DE FONGICIDES A BASE DE
BENZIMIDAZOLE EN SYNERGIE AVEC LE MERCURE.

L'un des buts de l'étude était de déterminer le devenir de l'Amino-2-Benzimidazole (A2B = produit final de dégradation, stable dans l'environnement, de divers fongicides) sous l'influence de divers agents de traitement des eaux recyclées : chlore et ozone. Dans l'optique d'une possible synergie entre micropolluants des eaux aboutissant à l'apparition de toxicités supplémentaires, l'interaction chimique et physiologique entre A2B et sels de mercure a été abordée. Dans une première phase seule l'action du chlore par l'intermédiaire de l'hypochlorite de sodium a été étudiée de façon détaillée.

I - RESULTATS ANALYTIQUES

Deux méthodes de dosage de l'A2B en solution aqueuse ont été mises au point : l'une par polarographie entièrement originale l'autre par colorimétrie.

2 - RESULTATS CHIMIQUES

Les produits formés en milieu acide et alcalin sont extraits par filtration, évaporés à sec et chromatographiés sur colonne. Les différentes fractions obtenues par chromatographie sont analysées par spectrométrie de masse à haute résolution. L'ensemble des produits identifiés est donné sur le tableau. Seules les synthèses du monochloro 5, A2B, du dichloro 5,6 A2B et de l'Azobenzimidazole ont été réalisées comme contretype à ce jour.

3 - RESULTATS BIOLOGIQUES

Le matériel biologique retenu pour cette étude est le cyprin doré d'élevage aisé et facile à trouver en grande quantité. La CL_0 (concentration létale 0) est supérieure à 100 ppm d'A2B.

La CL_{50} 24 h de l'A2B = 450 ppm

CL_{50} 24 h du CL_5 A2B : 80 ppm - CL_{50} 96 h du CL_5 A2B : 50 ppm

CL_{50} 24 h du dichloro A2B : 25 ppm - CL_{50} 96 h du dichloro A2B: 5ppm

La toxicité globale : 100 ppm A2B + 0,1 ppm de chlore → 50 % des poissons morts en 19 jours. Le métabolisme de l'A2B est aussi envisagé chez le cyprin. L'A2B et ses métabolites éventuels sont extraits par la méthode de KIRKLAND adaptée à ces composés. Le seul métabolite mis en évidence par spectrométrie de masse à haute résolution est l'hydroxy 5 A2B.

4 - INTERACTIONS A2B ET CHLORURE MERCURIQUE

CL_{50} 48 h de $HgCl_2$: 2 ppm

CL_{50} 48 h de $HgCl_2$ + 100 ppm A2B : 50 % des poissons meurent au bout de 8 jours.

20 à 50 % vivent encore 30 jours après.

Le mercure est dosé directement dans les différentes parties du poisson : chair, viscères, écailles (placées à cet effet dans des cruchons) par activation neutronique.

L'A2B prolonge la vie des poissons sans pour autant empêcher l'accumulation du mercure dont les teneurs, au contraire, sont fortement augmentées. L'A2B à titre préventif n'augmente pas la résistance du poisson à l'intoxication mercurielle; il semble même favoriser la fixation du mercure dans la chair.

L'A2B ne semble pas avoir d'effet curatif sur l'intoxication mercurielle.

5 - INTERACTIONS CHLORO A2B ET CHLORURE MERCURIQUE

50 ppm CL_5 A2B + 1 ppm $HgCl_2$ → tous les poissons meurent en moins de 24 h

9 ppm dichloro A2B + 1 ppm $HgCl_2$ → " " " "

Témoin 1 ppm $HgCl_2$ → 2/5 meurent en moins de 24 h.

CONCLUSION

L'A2B est transformé sous l'action de l'hypochlorite de sodium ou du chlore et conduit à une vingtaine de produits identifiés par spectrométrie de masse à haute résolution. Certains de ces produits de transformation sont beaucoup plus toxiques que les produits de départ. Le cyprin doré fixe facilement l'A2B qui est métabolisé en hydroxy-5 A2B. La voie métabolique est la même que celle suivie par d'autres espèces. L'A2B protège le poisson contre l'intoxication par le chlorure mercurique et cette protection semble découler de deux phénomènes : action spécifique de l'A2B sur le déroulement de l'intoxication et (ou) précipitation du mercure. L'action protectrice globale ne s'observe que lorsque l'A2B est ajouté en même temps que le mercure dans l'aquarium. Elle n'évite pas l'accumulation du mercure dans l'organisme du poisson.

PUBLICATIONSA paraître :

. "Recherche et dosage de l'Amino-2-Benzimidazole en milieu aqueux par polarographie". Y. ROCHE - D. CANTIN - J. VIGIER - A. BOUCHERLE
European Journal of Toxicology.

. "Fongicides dérivés du Benzimidazole. Réaction de l'hypochlorite de sodium sur l'Amino-2-Benzimidazole". Y. ROCHE - J. VIGIER - J.L. BENOIT - GUYOD - A. BOUCHERLE.

Bull. Soc. Pharm. LYON

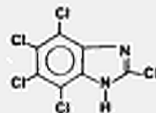
En préparation :

. Action de l'hypochlorite de Na sur l'Amino-2-Benzimidazole en solution aqueuse. Identification des molécules formées. Y. ROCHE - J. ULRICH - A. BOUCHERLE.

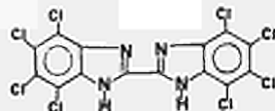
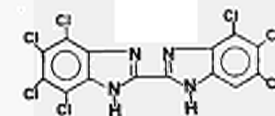
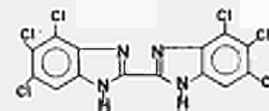
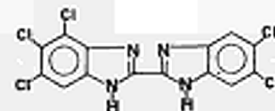
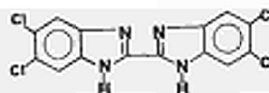
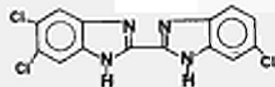
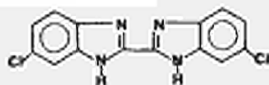
. Toxicité pour le cyprin doré des molécules formées par action de l'hypochlorite de sodium sur l'A2B en solution aqueuse. Métabolisme de l'A2B. Y. ROCHE - A. BOUCHERLE

. Toxicité du chlorure mercurique pour le cyprin doré. Interaction de l'A2B, du monochloro A2B, du dichloro A2B avec le chlorure mercurique.
Y. ROCHE - J. DIEBOLT - A. BOUCHERLE

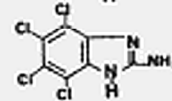
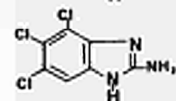
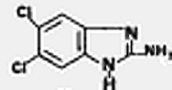
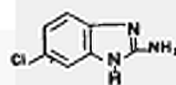
Dérivé du Benzimidazole



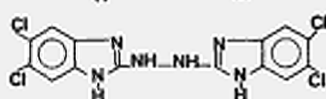
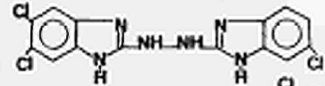
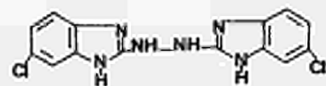
Dibenzimidazoles chlorés



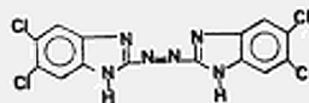
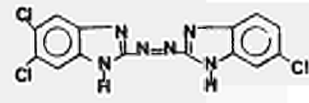
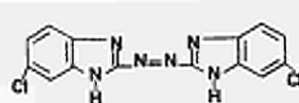
Aminobenzimidazoles chlorés



Hydrazobenzimidazoles chlorés



Azo bis benzimidazoles chlorés



Contracting party: SOCIETE NATIONALE DE PROTECTION DE LA NATURE
57, Rue Cuvier 75005 PARIS FRANCE

Contract number: 114 - 75 - 1 ENV. F

Project head: CENTRE D'ECOLOGIE DE CAMARGUE - CNRS
Le Sambuc 13200 ARLES
(Professor Ch. SAUVAGE, director)

Project title: A study of pollutants affecting the Camargue.
Pest control.

JUSTIFICATION

The important role played by the Camargue in maintaining the biological balance of both Europe and Africa is so well known that the region is the subject of much attention, both nationally (existence of the Réserve nationale and the Parc naturel régional de Camargue) and internationally (the Réserve nationale received the european diploma for nature conservation granted by the Council of Europe and is classified as a reserve of the biosphere within the framework of the Programme on man and the biosphere -MAB).

The Rhône river delta is not, however, free from pollution. Atmospheric contamination resulting from the industrial development of the Rhône valley and the Golfe de Fos is not yet a major problem but aquatic pollution is extremely serious. It is the result of the following practices:

1. The massive use of polluted Rhône river water (300 to 400 million m^3 per year) for crop irrigation. Rice fields are the major consumers of this water (35 000 to 40 000 m^3 per hectare and per year), since rice is grown on, depending on the year, from one third to one half of the cultivable land in the Camargue (20 000 hectares).
2. The extensive application of organic and mineral pesticides (several tons of active material per year).
3. The drainage of 30 to 40 million m^3 per year of polluted water into the étang du Vaccarès, containing polluting agents from the Rhône river and agricultural practices.

Rhône river pollutants: urban and industrial waste: hydrocarbons, detergents, organic matter, organo halogenated compounds, heavy metals (mercury, chrome, cadmium).

Agricultural pollutants:

Insecticides: lindane, numerous organophosphorous compounds such as parathion and arsenic. Herbicides and algicides: molinate, propanil, chlorophenoxy compounds, carbamates, copper.

The objective of the Centre d'écologie de Camargue (CNRS), in collaboration with the Réserve nationale (SNPN), within the framework of the programme on environmental research is centered around three essential points:

1. The study of the contamination of natural aquatic environments: inventory of pollutants and the determination of their origins as well as their transfer through food chains.
2. The study of the organisation of the rice field ecosystem and the consequences of pesticide use.
3. Research for alternate methods of pest control.

RESULTS

1. Contamination of natural aquatic environments (Vaccarès)

Inventory: traces of alpha and gamma HCH and of polychlorinated biphenyls coming from drain water and residues left by airplanes have been detected in the Vaccarès. Herbicide residues, found in drainage canals during application periods are not present in the Vaccarès. Traces of organochlorine insecticides (DDT, dieldrine), forbidden by french law, are found only at upper trophic levels (fish). As for hydrocarbons, their content in drainage canals is several ppm whereas this level is reduced to around a tenth of one ppm in the Vaccarès. The heavy metals, notably copper, lead and mercury are present in amounts varying from several half scores of ppb for copper, to several ppb for lead, and to just a few tenths of one ppb for mercury, when it can be detected. However, it is difficult to establish what percentage of these residues is due to the sedimentation of the delta and what percentage has its source in pesticides inputs.

Phenomena of biological concentration in the Etang du Vaccarès- Lindane

deposits from drain water	0,10 ppb
deposits from rain water	0,05 ppb
average content in the Vaccarès water (proving that there is decontamination by evaporation and codistillation)	0,01 ppb
content in silt (wet weight)	1 to 2 ppb
content in aquatic plants (wet weight) (filamentous algae and phanerogams)	2 ppb

content in molluscs (wet weight)	3 ppb
content in fish (wet weight)	6 ppb

- Heavy metals

Concentration factors in fish (wet weight) in comparison to water: copper and lead, 100 and 200; mercury, more than 1000.

2. Organisation of a rice field ecosystem and the consequences of pesticide use

From an ecological viewpoint, rice fields are temporary ponds which replace natural marshes that dry up in the summer, and consequently their biological activity is important at all trophic levels. The fields are subjected to systematic applications of insecticides and herbicides in may-june and occasional insecticide treatments in july-august. Studying the kinetics of pesticides reveals that they are so transient in water that they are an important polluting factor only during their application. Thus the shock effect of both insecticides and herbicides is serious on invertebrates, while the risk of biological concentration is limited despite the persistence of certain elements in the soil. The transfer of pesticides in drain water represents only a very small percentage of the original input (1/80 for lindane; 1/12 for molinate).

The study of the rice paddy ecosystem takes into account these important factors: the overall hydraulic situation, the physico-chemistry of the environment, the nature and fate of the pesticides used, their effect on the biocoenosis; the study of adventitious algae and phanerogams, and the zooplankton populations. Further studies, still fragmentary, deal with the soil microbiology, the chironomides and the transfer of nitrogen and phosphorous (showing that there is little risk of eutrophication by the transport of chemical fertilizer residues).

3. Research for alternate methods of pest control

The objective is to demonstrate the uselessness of certain agricultural practices and pesticide applications.

It is possible to affirm that:

- The amount of irrigation water could be reduced
- The use of lindane in association with parathion is neither effective nor justifiable
- Molinate, which is toxic for invertebrates but helps the proliferation of algae when applied in the dose recommended for agricultural purposes is not the most efficient way of controlling adventitious

plants such as Echinochloa.

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1977

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-

Contractor: Istituto Italiano di Idrobiologia, Pallanza (Italy)

Contract n° : 041-74-1 ENV₁.CEE-III

Project Leader: Prof. Ettore Grimaldi

Title of project: Diffusione delle infezioni fungine in relazione al processo di eutrofizzazione: studi del contesto ecologico della infezione branchiale da miceti attribuiti al genere Branchiomyces a carico della popolazione di Alborella (Alburnus alburnus alborella) del Lago Maggiore.

In the last years freshwater biologists, but also public opinion, have been struck by an ever-increasing occurrence of diseases among fish populations in European inland waters. Mycoses and microbial infections seem to be the main responsables of this recent trend.

It is beyond argument that this increasing fish morbidity is in some way correlated with increasing water pollution. Therefore, the general problem of water pollution cannot be satisfactorily approached without paying adequate attention also to the emerging aspect of fish morbidity. We must particularly stress that some of these diseases were previously known only for artificial environments characterized by high fish densities and/or by temporarily unsatisfactory life conditions.

Infections caused by fungi belonging to the genus Branchiomyces (Phycomycetes Saprolegniales) were previously known as the cause of "gill rot" affecting carps in small eutrophic water bodies characterized by heavy organic load, algal blooms, and high summer temperatures. The first reports of Branchiomyces "gill rot" affecting populations of the small pelagic cyprinid Alburnus alburnus alborella (bleak) in natural standing waters of Northern Italy go back to 1964. Afterward, this disease was frequently observed in large and deep lakes of glacial origin located just at the South of the Alps causing impressive mass mortalities. In order to evidenciate the possible correlations between the disease and the main factors involved in the eutrophication process of these lakes, limnological and histopathological investigations were made on Lake Maggiore and its bleak population. During the period covered by this investigation (1975), two epizootics were observed, one during spring and one in summer. The first epizootic, not caused by Branchiomyces, was characterized by the same pathological picture skown in hatcheries by young salmonids and eels affected by the so called "gill disease", which is generally ascribed to the combined action of unfavourable environmental conditions and bacterial organisms. On the contrary, Branchiomyces was always recognized in histological sections of gills from diseased bleaks sampled during summer mortality.

Among limnological variables considered, concentrations of un-ionized ammonia in Lake Maggiore were found to be in obvious correlation with the occurrence of bleak mortalities (NH_3 values of 13 p.p.b. and 10 p.p.b., respectively in spring and summer episodes, against values of about 1-2 p.p.b. usually occurring, see Fig. 1).

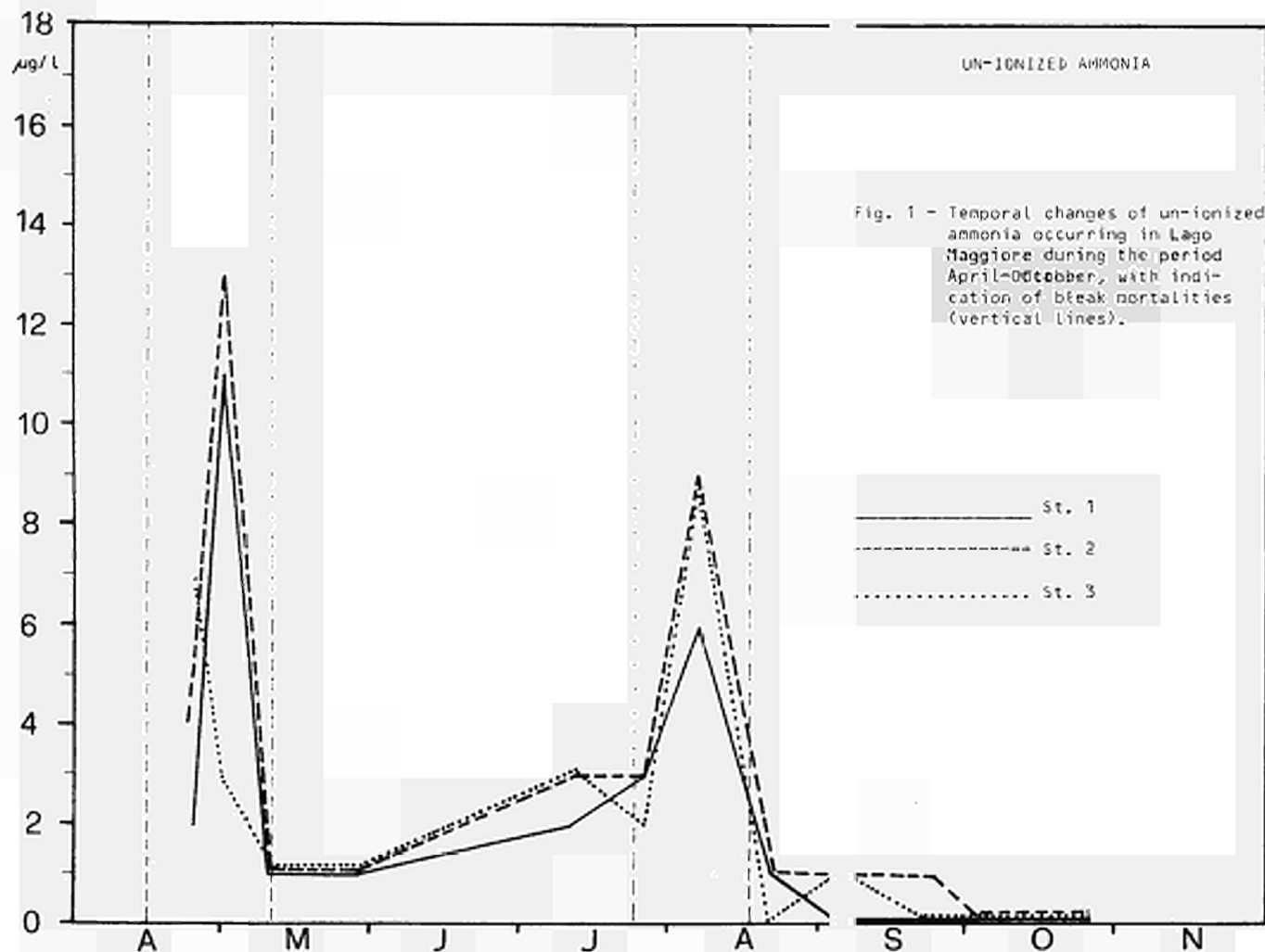
As far as we know, quantified evidences of causal correlations between limnological parameters and infectious diseases affecting natural fish populations living in large bodies of water were lacking. During the last years an increasing attention was paid by investigators to the pathogenic role of un-ionized ammonia (Burrows, 1964; Flis, 1968 a,b; Mayer and Kramer, 1973; Smart, 1976). Concentrations of NH_3 as low as 5-6 p.p.b. were considered able to alter epithelia of young trouts cultured for a few weeks in these conditions.

In our opinion, the main interest of this investigation lies on the demonstration of the pathogenic role of un-ionized ammonia at the level of fish population and in a large water body, that is in quite different environmental conditions than those indicated above. Furthermore, our results do not seem to point out a direct toxic action of un-ionized ammonia, but rather its "noxious stimulus" (Snieszko, 1974) in predisposing the gill apparatus to the attack of different pathogens, apparently bacteria in spring "gill disease", fungi in summer branchiomycosis.

It is well known that relative importance of un-ionized ammonia in aqueous ammonia solutions increases very rapidly with temperature and pH (Emerson, Russo, Lund and Thurston, 1975). In our case pH values well beyond neutrality were determined by high photosynthetic activity of dense phytoplanktonic populations. In coincidence with spring "gill disease", the relative importance of un-ionized ammonia was increased mainly by pH, whereas during summer branchiomycosis temperature also played a very important role.

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Contractor: C.N.R. - IRSA Rep. Sperim. Idrobiol. Applicata

Contract n° 042 - 74 - 1 ENV I

Project Leader: R. PASSINO

Title of project: Algal bioassays applied to the study of marine coastal waters for descriptive and provisional purposes

A marine algal bioassay for use in the European Communities countries was standardized. This bioassay, which on a general basis is quite similar to the "Marine Algal Assay Procedure Bottle Test" of the U.S. Environmental Protection Agency issued in December 1974, is intended primarily for use in the following general situations:

- 1) assessment of a receiving marine coastal waters to determine its nutrient status and sensitivity to change;
- 2) evaluation of materials, products and waste waters to determine their potential effects on algal growth in receiving waters.

Without describing in detail the methodology we would outline the most important points.

The test organisms selected are the chlorophicean Dunaliella tertiolecta and the diatom Phaeodactylum tricornutum. In order to verify the performance of the two test organisms in the cited general situations laboratory experiments were carried out:

- 1) The different methods for the measurement of algal growth were examined and compared: number of cells, optical density, dry weight, chlorophyll content by spectrophotometry after extraction or by direct fluorometry.
- 2) The linearity of the relationship between growth and nutrient concentrations was verified.
- 3) The two elements which act as the principal factors in the development of eutrophication in marine coastal waters which can be more easily controlled by the present technology are nitrogen and phosphorus. Algal growth experiments were developed in which the concentrations

of one of the two elements was fixed and the other varied. The variations of N/P ratio so obtained allowed to establish the optimal assimilation ratio for the two elements and the ratios at which one of the two acts as limiting factor.

The optimal ratio ranges from 6 to 6.5 in weight (about 13.7 to 14.8 by atoms) for Dunaliella and from 5 to 6 (11.4 to 13.7 by atoms) for Phaeodactylum, in agreement with results from other Authors (Ryther and Dunstan, 1971; Redfield, 1958).

- 4) The linearity of the growth response in media at different salinities (36, 24, 12 and 6‰) in relation to phosphorus concentrations in the limiting range was verified. For this purpose the two test algae were subcultured for long time for acclimatation in media of salinity ranging from 36‰ to 6‰. A lot of experiments was carried out for each salinity utilizing inocula obtained from all acclimatation subcultures.

The results show that the growth of Dunaliella is in linear relationship only with nutrient concentration and is not affected by the salinity neither of the test medium nor of the acclimatation medium. As a consequence Dunaliella could be used for the routine algal assay in a wide range of salinities of coastal areas. At the contrary Phaeodactylum at the minimum salinity investigated (6‰) shows an highly variable growth. At the highest salinities (36‰, 24‰ and 12‰) the growth was reliable and in linear relationship with the strength of the media. This organism should be used only in this range of salinities.

- 5) The toxicity of some heavy metals (Cu, Cd, Co, Ni) was also examined. The results show that Phaeodactylum is more sensitive to heavy metals than Dunaliella. The copper is the most toxic element on a weight basis.

The field application of the standard algal bioassay was carried out in 35 sampling stations located in the Ligurian sea in areas characterized by different types and levels of pollution. 12 other stations were established in the area around the Tiber river estuary. The re=

sults demonstrate the good sensitivity of the algal assay to discriminate among areas of slightly different trophic level and to identify sharply conditions of limitation.

In the figure are reported, as an example, the results obtained with Dunaliella in three stations characterized by different conditions of ecological situation. The station 1 is located in area completely unpolluted (Portofino Promontory) and the quality of the water could be considered similar to the typical pelagic mediterranean water. The growth of Dunaliella is in relation with the extremely poor nutrient concentration, in particular phosphorus that is to be considered as the primary limiting factor. Phosphorus limitation is indicated by the N/P ratio in the water and by the results obtained in the experiment with nutrient spikes.

The station 2 located in area characterized by weak nutrient pollution (west area of Genova gulf) evidences a higher algal growth and again the same condition of phosphorus limitation.

In the station 3, subjected to strong urban pollution (near Genova town) the results show very high algal growth, in agreement with nutrient concentration, and nitrogen limitation. This fact is in direct dependence of the N/P ratio lowering in sea water derived from the discharge of urban waste waters with low N/P ratio (Sawyer, 1971). The field application of the test clearly demonstrates that the most inshore mediterranean areas are characterized by phosphorus limitation. Nitrogen is the limiting factor only in the strong polluted areas; other elements were never found as primary limiting factors. The results of field application of the standard test indicate generally that for the control of eutrophication in mediterranean coastal areas it is imperative to remove phosphorus by waste waters.

On the basis of the whole laboratory and field experimentation, in line with the aim of the contract, it is possible to propose the application of the standard algal assay procedure:

- 1) to identify algal growth-limiting nutrients;
- 2) to determine biologically the availability of algal growth-limiting

ting nutrients;

- 3) to quantify the biological response to changes in concentrations of algal growth-limiting nutrients;
- 4) to evaluate the effects on marine coastal waters of waste waters discharges and to suggest the necessary treatments;
- 5) to determine also whether or not various compounds or water samples are toxic or inhibitory to phytoplankton.

Publication prepared within the contract.

Chiaudani G. e Vighi M.

Metodologia standard di saggio algale per lo studio della contaminazione delle acque marine.

Quaderni dell'Istituto di Ricerca sulle Acque. In press.

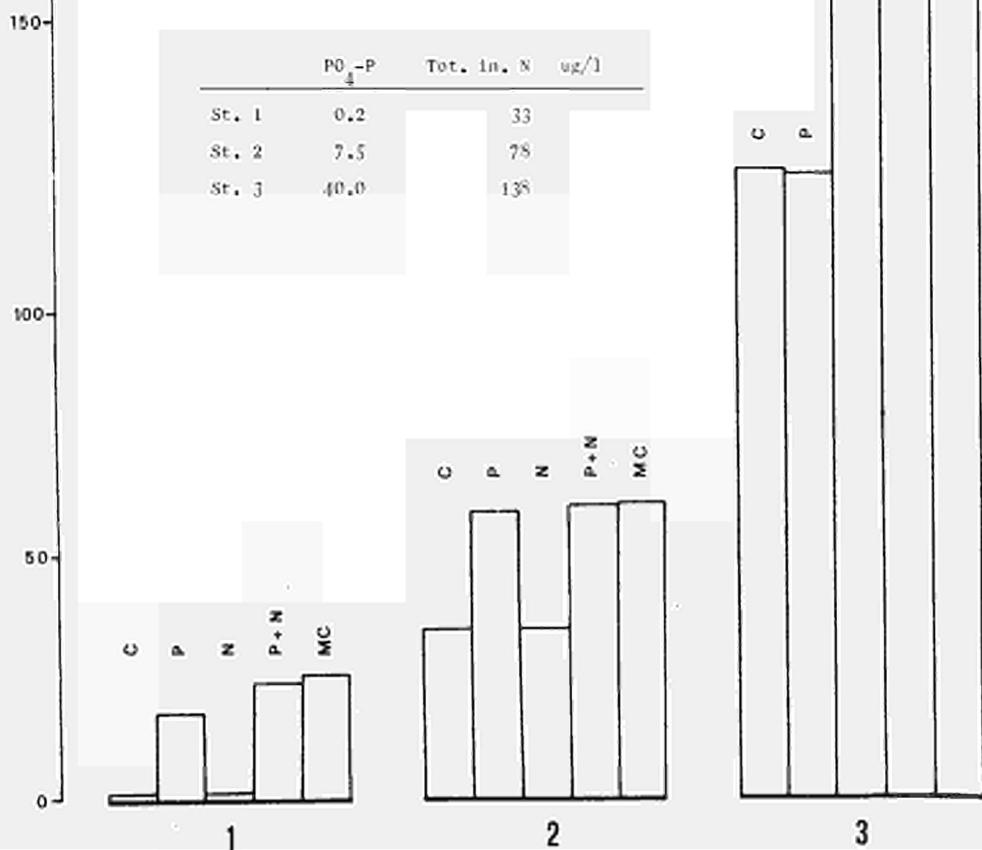
250 cells/l
x 10⁶

Figure 1

Results of spike experiments with *Dunaliella* in three stations located in Ligurian sea. It is shown the algal growth in the control (C) and in subsamples supplemented with phosphorus (P), nitrogen (N), phosphorus plus nitrogen (P + N) and complete medium (MC).

The concentrations of orthophosphate phosphorus and total inorganic nitrogen in the three stations were the followings:

	PO ₄ -P μg/l	Tot. in. N μg/l
St. 1	0.2	33
St. 2	7.5	78
St. 3	40.0	138



Contractor : Consiglio Nazionale delle Ricerche, Rome, Italy.

Contract n° 060-74-1 ENV I

Project Leader : B. Battaglia (co-investigators: L. Chieco-Bianchi, A. Colombatti and V.U. Fossato)

Title of project : Polynuclear Aromatic Hydrocarbons in Edible Molluscs and Their Oncogenic Properties.

The specific objectives of this study were: a) the determination of levels of carcinogenically significant polynuclear aromatic hydrocarbons (PAH), mainly 3,4-benzopyrene (BaP), in mussels (*Mytilus* sp.) collected in areas of the Laguna Veneta and the adjacent Adriatic Sea that are subjected to different levels of hydrocarbon contamination. This research was carried out at the Istituto di Biologia del Mare, C.N.R., Venice. b) The preparation of tissue extracts of mussels from the most heavily polluted zones and the determination of their oncogenicity in mice and rats. The animal studies were conducted at the Laboratorio di Oncologia Sperimentale, Istituto di Anatomia Patologica, University of Padua.

This simple scheme should be sufficient to give a basic picture of pollution, indicative of the level of contamination by PAH's in the Laguna Veneta, and to provide, by using biological and chemical indicators, the first background data to establish a carcinogenic index associated with water pollution.

Determination of polynuclear aromatic hydrocarbons in mussels.

The initial phase of this investigation involved the development and testing of various methods for the extraction, isolation, identification and quantification of hydrocarbons contained in mussel tissues as a contaminant and the preparation of a primary extract to be used as the putative carcinogenic agent in the animal studies.

The first phase in the preparative procedure, adopted for all subsequent uses, involved the saponification of the mussel tissues with alcoholic potassium hydroxide followed by an extraction of the tissue digest with n-hexane to recover the liberated hydrocarbons. This technique, unlike the more commonly used procedure of direct extraction of the tissues in a Soxhlet, is one of the most frequently used for the recovery of BaP, a widely recognized carcinogen known to be present as a contaminant in the tissues of marine animals.

For the analytical studies the extract was purified on a column of alumina, the hydrocarbons then separated by thin-layer chromatography using silica-gel as the medium followed by quantitative determination by spectrofluorometry of the BaP and perylene (Pe) fractions.

In the first half of 1975 we conducted preliminary determinations of BaP and Pe in several natural mussel populations in the Laguna Veneta and the adjacent Adriatic Sea. Following these studies we initiated a program of bimonthly analysis for BaP and Pe in mussels collected from ten stations. This monitoring, continued for eighteen months, has furnished the first background data on the levels of carcinogenic hydrocarbons in these bivalves, the culture of which is an extremely important sector of commercial fisheries in the Laguna.

The concentrations of BaP varied from 0.5 to 70.8 $\mu\text{g}/\text{kg}$ of wet tissue and that of Pe between 0.1 and 15.6 $\mu\text{g}/\text{kg}$. The highest recoveries of these compounds have been from tissue of Mytilus collected in waters adjacent to the industrial port area and within the cities of Venice and Chioggia. The lowest levels of BaP and Pe were encountered in mussels from the Adriatic Sea and in the central basin of the Laguna, farthest from pollution sources. If we consider the level of contamination of mussels at various distances from pollution sources, a gradient can readily be demonstrated. These results are in agreement with the distribution of oil pollution in the Laguna, as determined previously on the basis of the aliphatic hydrocarbon content of the resident mussels.

With notable exceptions, seasonal maxima for BaP and Pe content occurred in January, minima in May and July 1976. Probably these differences are related to a higher mean effective pollution load in winter as well as to the reproductive stage of these organisms, which have in this area an extended period of spawning during spring and summer.

The range of concentrations of BaP and Pe reported for mussels from the Laguna Veneta do not differ markedly from those measured in similar areas throughout the world and probably they do not represent a serious hazard for man. It should be pointed out, however, that molluscs accumulate in their tissues other compounds and elements with carcinogenic activity, such as DDT, PCB's, Cd and As, the sum effect of which can only be evaluated by additional chemical and biological tests.

In vivo carcinogenesis assay

To determine the potential oncogenicity of mollusc tissues contaminated with hydrocarbons, crude tissue extracts were prepared using mussels collected in the fall of 1973 from the most heavily polluted zones in the Laguna. Various doses (ranging from 50 to 3,000 μg) of these extracts, solubilized in olive oil, were inoculated subcutaneously in newborn CBA and RFM/Un mice (0.1 ml), as well as in newborn Wistar/Fu rats (0.3 ml). Positive controls were inoculated with methyl-cholanthrene (MC), 17 μg in 0.1 ml olive oil for mice and 150 μg in 0.3 ml for rats. Negative controls received the vehicle (olive oil) alone.

The observation period was 18 months for mice and 24 months for rats. The following parameters were considered: acute toxicity, as well as development, latency and histological type of neoplasias.

No substantial differences in survival rate were detected among the

experimental animals, except for the groups injected with MC.

Nearly 100% of CBA mice injected with MC had tumours, mostly fibro- and rhabdomyosarcomas at injection site, and multiple pulmonary adenomas. No tumours were observed in 26 olive oil treated mice. A total of 20 out of 144 (13.8%) mice in the groups treated with mussel extracts developed tumours. Of some interest was the appearance of 5 leukemias in mice treated with the highest dose.

RFM/Un mice showed on the whole no statistically significant differences between negative control groups (33 mice) and the experimental groups (106 mice). However, a significant increase above control levels ($0.1 > p > 0.05$) was detected as far as lung tumours were concerned.

Few tumours of several histological types were observed in the 69 Wistar/Fu rats injected with three dose levels of mussel extract, the total tumour incidence being similar to that of the negative control group. 27 out of 37 rats receiving MC developed soft tissue sarcomas at the injection site.

In conclusion, the above experiments suggest that crude extracts of mollusc tissues administered by single s.c. injection to newborn mice and rats have a weak oncogenic effect. However, before definite conclusions on the oncogenic potential of these extracts could be drawn, further experiments are required by using various treatment schedules as well as different extracts of mussels from polluted and unpolluted areas.

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Contractor : Ente Nazionale Risi, Milan (Italy)

Contract n°: O96/74/4 ENV I

Project leader: Dr. G. Corbetta

Title of project: Soil, water and rice fields pollution

The problems connected with the pollution of the irrigation and drainage water of the rice fields located in the Po Valley, between the Dora Baltea, Ticino and Po rivers, have been, from 1971 through 1973, object of researches carried out by Ente Nazionale Risi, in cooperation with the "Associazione irrigua Ovest Sesia" and "Est Sesia" and with the Provincial sanitary and prophylaxis Laboratories of Vercelli and Novara.

The results of the physical-chemical and ichthyological analyses have been published by the Ente Nazionale Risi, while the results of the microbiological analyses are under print.

The actual researches have been carried out during 1974 and 1975, in cooperation with above organizations, about following problems:

1. Analysis of the irrigation and drainage waters during (April-August) and after (November-March) the rice cultivation season, to determine their general physical-chemical, ichthyological and microbiological characteristics and the contents of pesticides.
2. Analysis of heavy metals and pesticides in rice field soils, at depths of 0-20 and 40, 50 cm, before and after one year cultivation.
3. Analysis of heavy metals and pesticides in the various parts of the rice plant (roots, straws, paddy, brown and milled rice).
4. Calculation of the balance of heavy metals and pesticides in two rice fields, in connection with the contribution of irrigation and rain waters, and the removal by drainage waters and by harvesting.

Following results have been obtained:

Symptoms of pollution by industrial plants, by sewers and by chemical products employed in agriculture (fertilizers and pesticides) have been found in all irrigation waters. Waters containing drains from rice fields are

generally less polluted than those flowing in from not rice growing areas.

Anyhow, the content of organic and suspension matters and mineral compounds (chlorides, sulphates, phosphates) do not present a danger for the rice cultivation but contribute in improving the fertility of the soil. Some perplexity might arise in connection with the sometime high contents of phosphates and nitrogenous substances, which might provoke the eutrophication of slow running and stagnant waters.

All tested waters are fit for fish culture (carps, tenches), except a few samples found polluted by drains of textile industries, and only one containing rice field drainage waters.

The presence of heavy metals (manganese, chrome, copper, tin, nickel, lead, zinc, mercury, cadmium) is not constant but variable in doses ranging from a few thousandths of ppm to quantities not detectable by analytic methods.

Following pesticides have been found in irrigation and drainage waters: DDT, DDE, DDD, DCBP, Dieldrin, Heptachlore-hepoxy, Lindane, Alfa-beta-delta BHC, PCB, HCB, Metoxychlore, Phenitrothion, Ethion, Phentoate, Malathion, Methylparathion, 2-4 D, 2-4-5 TP, Drepamon, Proparyl and Ordram, in doses ranging from tenths to deci thousandths of ppb or of ppm for Ordram only.

No trace of following products have been found: Aldrin, Captan, Chlordan, Endosulphan, Endrin, Heptachlore, Diazinone, Dimethoate, Ronnel and Saturn (benthlocarb).

The concentration of heavy metals in rice field drainage waters has always been inferior to that found in ir

rigation waters, except for tin, whose presence in rice fields is due to the application of algacides.

Also the concentration of pesticides has generally been lower in drainage waters than in irrigation waters, due to their biochemical, catalytic, photolytic and microbiological degradation, excepting Ordram, used for the control of "Echinocloa" weeds in rice fields.

In autumn and winter the concentration of suspended matters, mineral salts, heavy metals, pesticides, etc., has generally been higher, due to the lower flow of the rivers, in consequence of snowfall in the mountains and a slower melting of the glaciers.

Microbiological researches generally showed an increase of the total microbial content in drainage waters (in comparison to the irrigation waters) and at the same time, a marked decrease of faecal microflora (streptococci, colonbacteria, salmonella, etc.).

In the soil there has not been any significant difference in the content of heavy metals before and after one year rice cultivation.

In the upper layer (0-20 cm) the presence of these metals has generally been higher than at higher depths (40-50 cm). Only very small doses of the pesticides DDD, DDE, DDT, Heptachlore, Dieldrin, Metoxychlore, Heptachlorehepoxy, 2-4-5 TP and PCB have been detected in the soil and generally in the upper layer.

Small doses of heavy metals have also been found in the rice plants, especially in the roots and in the straws, but no tin has been noticed.

The contents in the caryopsides were negligible and mostly placed in the outer integuments.

Also the pesticides DDD, DDE, DDT, Lindane, Alfa-Beta-Delta BHC, 2-4-5 TP have been found mostly in the roots and in the straws, at doses ranging from tenths to deci thousandths of ppm. The balance of heavy metals and pesticides in irrigation and drainage waters of both tested rice fields has been positive (contribution) for chrome, copper, cadmium, nickel, lead, zink, DDE, DDT, Dieldrin, Lindane, Beta and Delta BHC, Parathion, 2-4-5 TP and PCB, and in one rice field for mercury, manganese, Alfa BHC, Methylparathion and Ordram.

This balance has been negative (removal) in both tested rice fields for tin while the removal of mercury, manganese, Alfa BHC, Ordram and Propanil concerned only one rice field.

The heavy metals and pesticides brought in or removed from an hectare of rice field by the water were in any case very low and negligible for an agronomic and ecologic evaluation. Following pesticides have been found in the rains fallen during the 1975 rice growing season: DDE, DDT, Lindane, Alfa-Beta-Delta BHC, 2-4-5 TP, Ordram and PCB, in doses generally below one gram per hectare.

Only a few grams or fractions of gram of heavy metals and hundredths or thousandths of gram of pesticides are removed by the caryopsides, as the roots and the straws remain in the rice field.

Literature about this argument:

G. Corbetta - L'inquinamento da fitofarmaci nelle risaie dei Comprensori irrigui dell'Est e dell'Ovest Sesia. "Atti Giornate Fitopatologiche 1975" - Torino.

G. Corbetta, M. Leonzio - Indagine sulle condizioni ecologiche delle risaie. Il Risicoltore, n. 6, 7, 8 - 1977.

P. Torazzo, L. Vietti, M. Carello - 2, 4, 5 TP e altri composti fenossialcanoici presenti nell'ambiente risicolo. - Il Dal 1971 al 1974. Il Riso, n. 2 - 1977.

Contractor : Università di Torino
Ist. di Fisiologia e Chimica biologica

Contract n° : 108-74-1 ENV I

Project Leaders: Prof. L. Giulio, Prof. E. Meda

Title of project : Chemoreceptors of the lateral-line organs in
Xenopus laevis : Sensitivity changes under various
conditions.

The first experiment dealt with the effects of exogenous prolactin on some histological features, metabolic processes and certain ionic balances in hypophysectomized *Xenopus*. The following results were obtained :

- (a) In intact animals the increase in the discharge rate runs parallel with the rise in concentration of the stimulating solution, the threshold being 10 mM.
- (b) In hypophysectomized animals the concentration threshold is considerably higher than observed in intact animals ; moreover, a weak response is evoked by whatever concentration.
- (c) In prolactin-treated hypophysectomized animals the response to 10,25 and 50 mM KCl concentrations is identical to that evoked in intact animals, whereas for the highest concentration (100 mM) it is lower than in intact animals.

Prolactin treatment-which is of consequence in the adaptation of the animals to environmental changes and to variations in ionic balance-clearly succeeds in counteracting, for the most part at least, the effects of hypophysectomy.

In the second experiment changes in the level of endogenous prolactin were induced experimentally in progressively desiccated toads, which would mimic the conditions prevailing in their natural habitat. The possibility of a parallel modification of chemosensitivity was examined in three groups of animals : "dry" animals ; "dry" animals returned to water for 0+3 days ; "dry" animals returned to water for 5+9 days.

In order to discover whether the effects observed in the present experiments were caused by endogenous prolactin, a cytological investigation was carried out (in parallel with electrophysiological experiments) to detect the variations in the cytology of the cells in the pituitary gland which are presumed to produce prolactin, or a prolactin-like hormon; these cells are located in the posterior part of the distal lobe.

Results

- (a) The chemosensitivity of the neuromasts of toads kept under dry conditions (group I) was definitely reduced : the discharge rate in water and in progressively increasing KCl concentration was not significantly different. Cytological pattern of the cells presumed to be prolactin-producing suggests that the release of this hormone is limited.

- (b) An analogous situation to that displayed by normal animals is found in group II, toads returned to water for 0 + 3 days. Accordingly, the cytology of prolactin-producing cells is little changed, twenty-four hours after returning the toads to water.
- (c) Toads which have been in the water for 5 + 9 days show a quite different situation : application of KCl solutions of increasing concentration is paralleled by a tonic increase in discharge rate of neuromasts. This means that a normal chemosensitivity can be restored by returning toads to water for at least 5 days. The cytological pattern of the prolactin-producing cells of the toads which have been in the water for 7 days is quite similar to that of control animals, living in the aquatic environment : the cells are smaller and stain less readily, because at this time prolactin is produced and released at a continuous rate.

Conclusions

The first series of experiments showed that, when prolactin is given to subtotally hypophysectomized toads (which cannot produce their own prolactin) the normal chemosensitivity of the neuromasts can be maintained. Considering the alterations in the cytology of the pituitary gland which governs, more or less directly, the other structural and functional changes, and the electrophysiological results of the II series of experiments, it seems reasonable to suggest that the encephalic centres (in particular the hypothalamus) which regulate the activity of the pituitary gland are involved in the slow and gradual restoration of neuromast chemosensitivity in *Xenopus Laevis* and, more generally, in the adaptation of these animals to environmental changes of their natural habitat.

Contractor: Association EURATOM-ITAL, P.O. Box 48, Wageningen,
The Netherlands.

Contract No.: addendum to No. 094-72-1 BIO N

Project leaders: M.J. Frissel, J.H. Becking.

Title of project: The influence of nitrogen compounds and correlated
elements on eutrophication in surface waters and their
presence in deepground water (drinking water).

Nitrogen is one of the most important growth limiting substances, either at deficiency or at excess levels. When certain conditions are fulfilled nitrogen limits therefore e.g. the eutrophication of surface waters, but also the crop production.

Eutrophication indeed is not the only way in which its hazardous effects show up; toxification by high levels of nitrate in drinking water and vegetables, air pollution by the emission of N_yO_x compounds and possible decomposition of the stratospheric ozone layer by N_2O are other effects. It is therefore not surprising that an enormous amount of research is carried out on nitrogen. Examples are: nitrate leaching studies, studies on volatilization of ammonia from manure and fertilizers followed by wash out, fertilizer efficiency studies, preparation of N-balances, surveys of surface and ground water, studies on biological nitrogen fixation and studies on biological transformations of nitrogen compounds in soil. Although these processes are usually studied as separate processes they are in fact highly correlated to each other. An increased fertilizer efficiency will probably reduce both the leaching of nitrate and the emission of N_yO_x compounds into the atmosphere.

An increased biological nitrogen fixation will reduce the need for fertilizers and therefore possibly reduces the leaching of nitrate. No certainty exists on this point; it might be that the increased biological fixation also results in an increased leaching of nitrate, this will depend on the mineralization and immobilization characteristics of the organic material produced.

The purpose of the simulation model which was developed under this contract is combining the results of the various studies on the behaviour of nitrogen in soil.

The model developed so far, takes into account the following processes: mineralization and immobilization of nitrogen compounds in soil, transformation of ammonium in nitrates, leaching of nitrates, fixation of ammonium ions on clay minerals and volatilization of ammonia from the soil surface.

The processes are described by different equations which are solved by a special numerical method, usually called mathematical simulation. Input parameters were derived from different separate studies, such studies were also used to verify the different submodels.

Validating of the whole model requires simultaneous measurement of many parameters, such as rainfall, nitrate concentration in soil moisture, nitrogen in soil organic matter, active biomass in soil, uptake by the crop. This study could not be carried out under the considered contract, but is in progress as part of the present programme of the Association. Separate attention has been paid to the related (see above) eutrophication problem. At present it is well established that eutrophication of surface water is primarily caused by biomass production of algae, of which the blue-green algae form a substantial part. The periodic mass development or bloom of algae is correlated particularly with the presence of compounds of phosphorus and nitrogen. For the lakes studied the high phosphate level results from phosphate rich effluents, the high nitrate level from a combination of nitrate rich effluents and biological nitrogen fixation. A classification of the algae occurring in surface waters (e.g. the Veluwe lake) of the Netherlands according to species or genus rank was worked out as far as possible, in order to obtain an insight in the chronological events of the eutrophication process. In unpolluted lake water particularly green algae of the genus *Scenedesmus* (Protococcales) are abundant, but in the course of the eutrophication process these algae are gradually replaced by species such as *Aphanizomenon flos aquae* (L.) RfIs., *Anabaena flos aquae* (Lynghb.) Brb., *Nostoc* species and some other *Anabaena* species. Very polluted water mainly contains *Oscillatoria* species with as a dominant species the blue-green algae *Oscillatoria agardhii* Gomont. The results of this study will be published in due time by the "Rijksdienst voor IJsselmeerpolders" (Ir. G. Berger).

With regard to nitrogen, polluted water frequently contained more $\text{NH}_4\text{-N}$ (typical values between 1 and 2 mg/l) whereas the NO_3 and NO_2 concentrations remained relatively low. According to the available data, however, for

its nitrogen requirements *Oscillatoria agardhii* apparently lives saprophytically in the lake water and probably obtains its nitrogen from lysis of other algae cells or preceding algal or bacterial associations. The growth of such associations and therefore also of the nitrogen-fixing blue-green algae in surface waters is stimulated very considerably by phosphate from phosphate rich effluents (superphosphate dressing utilized in agriculture; polyphosphates derived from synthetic soaps and detergents in sewage effluents from towns).

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Contractor: Organization for Applied Scientific Research (TNO), Delft

Contract n^o: 110-75-1 ENV N

Project leader: Jan Kuiper

Title of project: Exploratory investigations into pelagic "microcosms"
subject to environmental stress by pollutants.

Introduction

Interpretation of the results of traditional toxicological laboratory experiments with single species in terms of environmental significance is difficult. To bridge the gap between laboratory and field conditions, plastic enclosures housing natural plankton communities have been used to study the ecology of freshwater and marine plankton under semi-natural conditions (Menzel and Case 1977).

The main objective of the study reported here was to find out whether the method is suitable for investigating the influence of environmental stress on a marine plankton system. In this framework we performed three experiments in 1975. The primary aim of the first and second experiment was to determine the variations occurring in duplicate bags (Kuiper 1977a). In the third experiment the structure and function of a plankton system was studied using mercuric chloride as a model pollutant, of which a single dose of 5 ppb was added (Kuiper 1977b).

In each of the three experiments, four plastic bags were filled with about 1400 l of North Sea coastal water and the development of phyto- and zooplankton and decomposers was monitored for 4-6 weeks, together with a number of physicochemical parameters.

Development of phytoplankton and decomposers

In each experiment the phytoplankton developed in the same way. Fig. 1 is shown as an example. A few days after the bags were filled, the phytoplankton began to grow. A maximum was reached, after which algal biomass declined. This first bloom was always generated by diatoms. The concentration of several nutrients decreased, indicating that the growth of the first bloom was probably limited by a depletion of nutrients. The bloom of diatoms was followed by one of microflagellates, which also passed through a maximum and declined. In two experiments the second bloom was followed

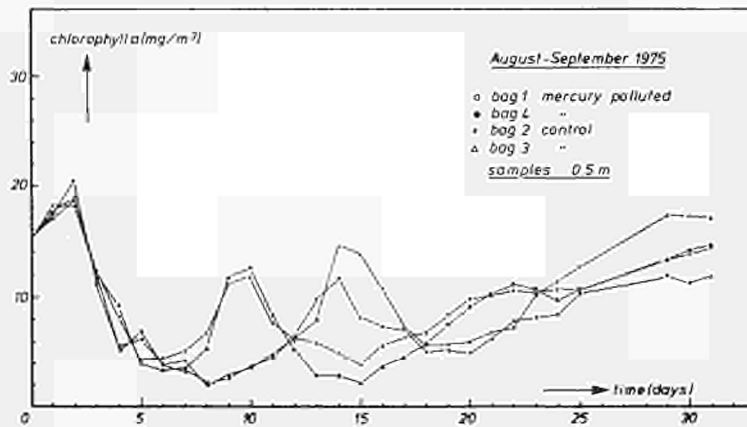


Fig. 1: Chlorophyll a concentration, at a depth of 0.5 m in mercury-polluted bags and non-polluted controls.

by a third. The fact that the first peak is followed by a second, or even a third, indicates that the organic matter is mineralized and recycled in a "natural" process.

Calculated growth and mortality rates of the algae in different bags revealed no significant differences. The heights and species composition of corresponding peaks were practically the same. Small timeshifts do occur, but the pattern of development is the same under identical environmental conditions (cf Takahashi et al. 1975).

Five days after the addition of mercury in the third experiment, its concentration in the water decreased to below the detection level (0.3 ppb). The decrease is caused by adsorption of mercury on particles settling to the bottom. The addition of mercury had the following effects:

- a decrease in the growth rate of the phytoplankton as long as the mercury concentration was higher than 1.55 ppb.
- a delay of the second phytoplankton bloom. The delay is probably caused by a reduced mineralization rate. This result indicates that mineralization occurs partly in the sediment, because it is very unlikely that the very low concentrations of mercury in the water could inhibit the activity of decomposers.
- a change in the community structure of the second phytoplankton bloom.
- an increase in methylmercury concentration in the sediment towards the end of the experiment.

Development of the zooplankton

The major species found in the bags were calanoid and harpacticoid copepods. Fig. 2 shows as an example that these copepods develop in the bags from the nauplius (and probably from the egg) to the adult stage. The development patterns of the zooplankton exposed to the same environmental conditions in different bags are the same. The addition of mercury in the third experiment had no clear-cut effects on the growth of the zooplankton, although it seems probable that mercury retards the growth of two species (*Centropages hamatus* and *Temora longicornis*) (Fig. 3).

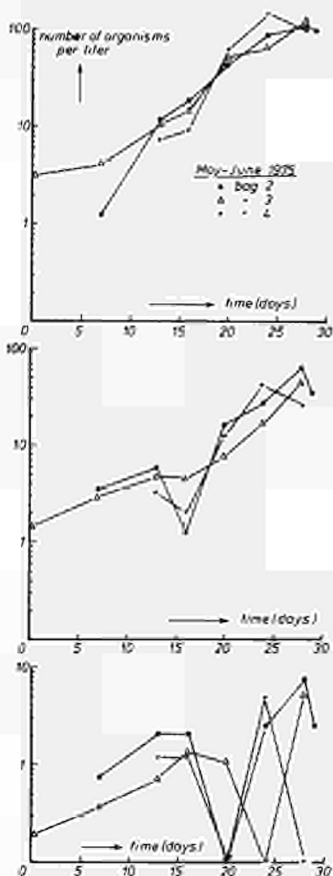


Fig. 2: Development of zooplankton (number of nauplii, copepodites and adult copepods per litre) during the second experiment.

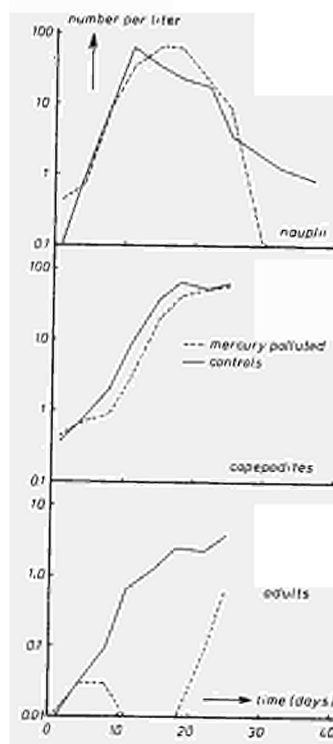


Fig. 3: Development of *Centropages hamatus*. Average from replicate bags (number of nauplii, copepodites and adults per litre).

Conclusions

Plankton communities from Dutch coastal waters, enclosed in plastic bags, during 4-6 weeks, show the same development patterns if the bags are filled simultaneously and exposed to the same environmental conditions. This result opens the way for toxicological application of the method. A single dose of 5 ppb of HgCl_2 in one experiment gave rise to several well-defined changes in the structure and function of the enclosed plankton system. This establishes the suitability of the method for assessing the influence of environmental stress on a marine plankton community. It seems that the plastic bag method may be regarded as a valuable tool in toxicological investigations.

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Acknowledgements

Thanks are due to my colleagues De Kock, Hoornsman, Roele, Schrieken, Van de Eikhoff and Van 't Groenewoud, who helped with the chemical, biological and statistical analyses. Acknowledgement is also due to other colleagues at Delft and to several colleagues of the Netherlands Institute of Sea Research to whom I am indebted for valuable help and advice. Finally I would like to thank the authorities of the Royal Netherlands Navy for their kind cooperation in providing experimental facilities.

Contractor : Rijksinstituut voor Drinkwatervoorziening, 's-Gravenhage

Contract n° : 111-75-1 ENV N

Project leaders : Drs. W. Slooff, Ir. B.C.J. Zoeteman

Project title : Toxicological aspects of organic compounds in drinking water with emphasis on toxicity monitoring based on detection of fish respiration

1. As a result of contamination of water resources with toxic organic pollutants and the increased need to use these waters for the production of drinking water, the study of the toxicological aspects of individual substances and mixtures of organic water contaminants has become a matter of high priority.
2. Although the concentration of toxic compounds and their potential toxicological effects can often be most easily detected in the raw water, it is generally felt that the toxicological evaluation of drinking water needs to be undertaken as a starting point from which the most important sources of tapwater contamination can be deduced and as far as possible subsequently eliminated.
3. For 21 selected organic tapwater contaminants literature data on toxic effects have been compiled and evaluated.
4. Among the approx. 400 organic compounds, identified in drinking water in the world, a number of halogenated low molecular organic compounds are found with the highest frequency and at the highest concentrations. Particular the haloforms, such as chloroform, and trichloroethylene, both weak carcinogens, have been frequently detected and maximum levels above 100 µg/l have been reported. Some moderate carcinogens like tetrachloromethane have been regularly detected at levels of approx. 1 µg/l or less while strong carcinogens such as vinylchloride and 3,4 benzo-pyrene seem to be present in drinking water only incidentally at levels of possible health significance.

5. In general insufficient data are available on the occurrence in drinking water of carcinogenic volatile compounds, such as some haloforms and halogenated alkenes, although it is known that these compounds can be present at relatively high levels in tapwater, derived from surface water as well as groundwater.
As these compounds can be measured rather easily on a routine basis it is recommended to carry out a systematic survey on their presence in all major water supply systems in the countries of the European Communities in the near future. The results of such an inventory will enable appropriate short term counter measures to reduce effectively the levels of these carcinogenic halogenated compounds in European tapwaters.
6. For a number of potential carcinogenic compounds, which have been detected in drinking water at levels of approx. 1 µg/l or more, insufficient toxicological data exist. In this respect particularly halogenated ethanes and alkenes as well as a number of chlorinated ethers merit further study.
7. Among the compounds for which massspectra and concentration estimates in drinking water are available, a relative large number of about 100 compounds exists for which the identity could not yet be established. International cooperation aiming at rapidly identifying the major compounds in this category is recommended.
8. The presence of possibly health significant quantities of organic compounds in tapwater can be related to two main sources: oxidative treatment using chlorination or ozonation and contamination of the raw water.

Besides the formation of haloforms and compounds like nitrotrichloromethane, chlorinated phenols and benzenes relatively little is known about the types and quantities of compounds which are formed under practical conditions during chlorination and ozonation for potable water supply. Further research in this area to identify the reaction products and to reduce the presence of those products in the final tapwater is of great importance for the futural design of water purification.

9. Significant causes of tapwater contamination are waste water discharges into surface waters, which are intended to be used for drinking water supply.

As these watersources can contain several hundreds of organic compounds, it is necessary in relation to possible deleterious effects on human and animal health, to use rapid monitoring systems for the detection of toxic effects.

10. Among the large variety of biological monitoring systems for detection of toxic levels of water contamination, the method using the critical flow technique and the system based on the detection of the respiratory pattern by means of dual external electrodes seem to offer good application possibilities to control continuously waste water or surface water near intakes for public water supplies.

In this way sudden acute toxic levels of chemicals in water can be detected within a period of approx. several hours.

In relation to the presence of potential carcinogenic organic compound -mixtures in tapwater the so-called Ames-test offers a valuable tool for periodic control of tapwater concentrates.

11. Tests with a biotoxicity monitoring system based on detection with dual external electrodes of changes in fish respiration patterns, such as breathing frequency, breathing depth and cough frequency, using rainbow trout (*Salmo gairdnerii*) as testorganism and chloroform and o-dichlorobenzene as toxicants, have illustrated the usefulness of such an early warning system.

These systems are detecting most sensitive directly acting toxicants such as certain metals and cyanides, which induce an increased cough frequency.

12. From theoretical estimations the system can be expected to indicate under optimal practical conditions accidents in river systems with chemicals like ammonia, copper, mercury, dieldrin, o-dichlorobenzene, endosulphan, hexachlorobutadiene, phenols and toxaphene.

High levels of haloforms up to approx. 1 mg/l will not be detected by this biotoxicity monitoring system.

13. More studies are necessary to evaluate the sensitivity of the tested and other biotoxicity monitoring systems for selected abundantly present aquatic pollutants.

It is recommended to carry out such studies by intensified research programs in the next years so that they may be generally available as soon as possible to control effectively waste water discharges and to enable a reliable operation of treatment systems for potable water supply from heavily contaminated sources.

Contractor: Fondation Universitaire Luxembourgeoise

Contract No.: CEE-FUL No. 034 - 74 - 1 ENV B

Project Manager: A. Schmitz, lecturer at the Fondation Universitaire Luxembourgeoise

Project Title: Hydrobiological study of the Semois river and influence of conifer plantation on aquatic macroinvertebrate fauna.

I. Spruce plantation influence on water quality

Comparative study of the aquatic macroinvertebrate fauna of 10 stations along streams of the Ardenne mountain mass under hardwood forest and 10 others under spruce plantation.

Samples are taken with the 300 cm² Hess cylinder for the benthic fauna, with the Mundie emerging trap for the eclosions, with the Wauters net for the derivative fauna and with the 13 cm² core for the digging fauna. The intensity of light which characterize the stations are evaluated by the Friend ozalid method and the hemispheric photography reading (Becker; large angular of 180°).

Inventories show that:

- under hardwood forest the vegetarian and detritiphage fauna is more important due to better lighting ensuring aquatic plant production and to edible dead leaves accumulation. There are especially bush trichoptera crustacea and ephemeroptera;

- under spruce, the digging fauna is the most important, especially chironomida, tipulida, ceratopogonidae and driopidae coleptera. This fauna does well withstand rapid flow variation, lack of light and water quality.

Moreover the fauna diversity quickly decreases with the increase of spruce plantation. In some cases (Anlier forest), the reduction of ephemeroptera populations seems to be due to light reduction whereas in Freyr forest the acidic character of the waters would be the principal responsible of such a decrease.

II. Pollutant charge and self-purification of the Upper Semois

The Upper Semois can be considered as a typical river having known a rapid degradation due to the increase of domestic pollution (Arlon town) and to river works (scraping and rectification).

The measurements demonstrate indeed that the mean discharge of waste waters, which are principally domestic ones, reaches 35 l/sec when river mean flow is only 10 l/sec. Daily variations of chemical parameters concentrations and fecal bacteria in the river downwards Arlon, reflect the typical daily activity of the residents: maximum of the urban discharge during the day, minimum during the night. Differences between minimum and maximum pollutant charge fluctuate by a factor of 10. These fluctuations of the boundary conditions at the entry constitute a determining factor for the choice of the kind of mathematical model used to follow the evolution of the pollutants in the river. Longitudinal dispersion phenomena of the daily pollution apex represent a process affecting largely the distribution of the pollutant in time and space. A special effort has been devoted to the longitudinal dispersion measurements of an inert tracer (Rhodamine WT) and to its interpretation. It was proved that, as in many other cases, dispersion was poorly described by turbulent diffusion models based on the Fich law. Therefore, we have developed a stochastic dispersion model which allows, by adjustment of two variables, to satisfactorily describe the observed concentration profiles. This model has been furthermore applied with success to the description of the evolution of total nitrogen and ammonia in the river. Moreover, we have at our disposal the spatial and temporal evolution of a great number of chemical and biological parameters in the river. Those measurements would be later modeled when more complete data regarding kinetic of their evolution will be gained. They constitute up to now a bank of interesting data, especially for the definition of the projected waste water treatment system of Arlon.

Contractor : Rijksuniversiteit Gent
Contract n° : 099-75-1 ENV B
Project Leader : Prof. Dr. A. COTTENIE
Title of project : Effects of mineral water pollution : ecological response and controlling techniques

1. Ecological test

In a first stage water- and bankplants were sampled in waterways with different mineral load. Their mineral composition was determined after identification of the sampled plants. This gives informations concerning the uptake of nutrient elements e.g. Ca, Mg, Na, K, PO₄, NO₃ and heavy metals Pb, Zn, Cu, Cd, Cr, etc.

The ecological study of water- and bankplants (identification and spreading) can be an identification for the pollution of waterways. In ponds, more or less ecologically in equilibration, a quite large variety of water- and bankplant species were found. In waterways and canals on the other hand, some species of water- and bankplants had disappeared on many places and this resulted in a more uniform plant growth. Only some clean waterways (used for the supply of drinking water) show an analogous ecological equilibration as the examined ponds. There was a striking difference between the waterplants in lakes and those in waterways. One species was found in both biotopes namely *Myriophyllum specatum* L.

Concerning the chemical composition there is a marked difference between water- and bankplants. The last ones contain generally lower levels of mineral components (major and trace elements). In comparison with the normal content of heavy metals in plants, waterplants contain high to very high amounts. Typical waterplants reflect clearly the mineral composition of the sediments in which they are growing. In this way different contents of nutrient and trace elements were found in the species, especially those growing in polluted waterways. Obviously, some plants could be maintained due to an adaptability, based on the great variability of their composition,

resulting in a biased plantgrowth.

2. Water-sediment studies

a. Water

The pH of most surface waters varied between 7 and 9, while groundwater had a lower pH. Heavy metal contents in surface and groundwater are usually very low. Iron and manganese concentrations are normally in the ppm range, while for the other metals ppb concentrations are found. Sample preparation (analytical procedures) has a great influence on the results and acidification e.g. results in high concentrations, due to the release from suspended particles.

Waterways heavily loaded by domestic wastewater show a marked anaërobic character in which lack of dissolved oxygen, high contents of suspended matter (turbidity), make life of fish and plants (macro-biota) impossible. These reducing conditions increase the Fe, NH_4^+ and PO_4^{3-} contents in the water. The same anaërobic trend was observed in rivers and canals, but to a lesser extent. The lower NH_4^+ and PO_4^{3-} values were caused by the dilution effect and eventual aërobic decomposition (dissolved oxygen concentration increased by better aëration and mixing). The total salt contents of some canals (Na, Cl^- , K^+ , Mg^{2+} and SO_4^{2-}) is quite different from that of rural waterways e.g. sea canals and drainage canals.

b. Sediments

The running water in rivers, waterways and canals contains suspended matter which settles slowly and forms the sediment. Riversalt and suspended matter influence the physical and biological properties of the surface water. The chemical aspects of silt-water interactions, adsorption phenomena, were examined. The characterization of the silt-structure was also studied.

- Content of heavy metals (extraction with HNO_3 0.5 n ; pH = 0.5)

In comparison with soil, sediments show very high contents of heavy metals. Extremely high values (195 ppm Cr) are not a natural phenomenon, but a result from human activity. Fe, Mn, Zn, Cu, Cr and Pb are the main metal ions, showing a heavy enrichment in the upper siltlayer. The accumulation of these metals is also closely correlated with the organic material content of the silt.

- *pH-H₂O and pH-KCl of riversediments*

Only small differences between pH-KCl and pH-H₂O were observed in all samples, showing that the silt complex is saturated with basic cations. Most soil sediments have a pH between 7 and 8, while lower pH values were found in ponds (4 to 7).

- *Contents of major elements (Na, K, Ca, Mg, P₂O₅)*

Very high contents of major elements were found in riversilt in comparison with soils.

- *Cation exchange capacity (C.E.C.)*

The C.E.C. is closely correlated with the organic matter content in riversilt and varies between 2 and 36 meq per 100 g.

- *Redoxpotential and sulfide contents*

Overloading (organic pollution) reduces the solubilized oxygen concentration, so that the whole waterway becomes anaerobic. In these conditions reduced ions like Fe²⁺, Mn²⁺, NH₄⁺ etc. can diffuse in the water. The rate of reduction was determined by Fe²⁺, S²⁻ and redox potential measurements on fresh and dry sediments. More or less ecologically equilibrated ponds with variable plant growth, gave averages of 4.5 ppm S²⁻, while in rural waterways and canals respectively 13 and 71 ppm S²⁻ were found (dried sediments). It is worth mentioning that heavy metals, which are generally toxic for plants, are immobilised by sulfides.

- *Mobility of trace elements in sediments*

Especially Mn, Zn, Co and Ni are the more mobile elements, while Cu, Pb and Cr are rather immobile. Mobility of trace elements in riversilt plays an important role with regard to plant uptake and influence of pH fluctuations on the interaction sediment-water. Hg contents were generally below 1 ppm. Adsorption and desorption phenomena of silt were studied on a laboratory scale.

3. *Immobilisation of mineral pollutants in aqueous solutions*

Case study with the element Zn. The removal of Zn from an aqueous solution was studied by means of the pH effect, complexation and precipitation with

lime, phosphates, silicates etc.

4. Summary and conclusions

The survey of the presence, distribution and mineral composition of water- and bankplants, sampled in a great variety of surface waters (ponds and waterways) showed a clear response of the kind of species and their Ca, Na, K, Mg, P_2O_5 and metals contents towards the typical growth conditions. Bankplants contain considerably lower amounts of mineral components than typical waterplants, though the latter are also quite different from the normal plants. In view of a more fundamental study of the factors which determine the uptake of inorganic components, the following sediment characteristics were considered : concentration of heavy metals, concentration of major elements, pH H_2O and KCl, C.E.C., redox potential, sulphide content, mobility and the form in which heavy metals are present in the sediments, namely as free ion, adsorbed on the sorption complex or complexed with organic matter. The saturation of the adsorption complex of the sediments is practically complete (pH H_2O nearly equal to pH KCl), which means that the sediments constitute a very rich "pool" of (heavy) metals. However, because of the neutral or slightly alkaline pH, these metals are rather strongly retained on the complex. However, relatively small changes of pH (decrease), redox-potential or organic matter content, may cause a dissolution of quite important quantities, becoming available for bank- and waterplants.

Contractor : UWIST, Department of Applied Biology,
King Edward VII Avenue, Cardiff CF1 3NU - UK

Contract n° 028-74-1 ENV UK

Project Leader : Prof. R.W. Edwards

Title of project : Chemical and biological studies of the river
Cynon, a tributary of the river Taff, South Wales

The river Cynon is a tributary of the river Taff in South Wales and is polluted by sewage, suspended solids or toxic wastes for most of its length.

Following a study by Hughes (1975)*, which established the nature of invertebrate communities and the water quality of the river in 1971 and 1972, the present work was undertaken to assess any changes which had occurred in the subsequent two year period. Some changes were anticipated as certain toxic effluents from the Phurnacite Works, Aberaman, which drained into the river during Hughes' survey and the early part of this study, were diverted into the trunk sewer in December 1974.

Major water quality parameters and invertebrate and fish distributions were monitored in 1974 and 1975. During this period, water quality improved slightly in the region of the Phurnacite Works and trout were recorded below the Works for the first time since research on the river began. However, invertebrate communities showed very little sign of change in this region.

In the middle and lower reaches as a whole, the invertebrate community appeared very stable, consisting chiefly of oligochaetes and Asellus aquaticus. Upstream of Aberdare, the communities were less stable. In the headwaters, there was a diverse community composed largely of ephemeropterans, plecopterans and trichopterans. Between this region and Aberdare, there were communities transitional between the two types already mentioned. At Penderyn, inputs of limestone dust affected the nature of both invertebrate and fish communities.

Since the study of Hughes, both water quality parameters (particularly ammonia, suspended solids and sodium) and fish distributions have shown signs of an improved situation in the lower reaches of the river. Invertebrate trends have been less clear, but suggest a worsening situation in the upper-middle reaches, where sewage pollution is an important factor.

* B.D. Hughes. A comparison of four samplers for benthic macro-invertebrates inhabiting coarse river deposits. Wat. Res. 9, 61-69, 1975

B.D. Hughes. A study of a polluted river with emphasis on the benthic invertebrates. Ph. D. thesis. Univ. of Wales.

Contractor : Imperial Chemical Industries Limited, Brixham Laboratory

Contract No : 073-74-1 ENV UK

Project Leader : Dr P N J Chipperfield

Title of Project : The Effect of Four Industrialised Estuaries upon
the Coastal Waters of North-Eastern England

Introduction

In 1970, the Brixham Laboratory of ICI began a multidisciplinary study of the environmental impact on coastal waters of the contaminated waters discharged from the Tees Estuary. During 1974-75 the grant of an EEC contract permitted an extension of the study to cover the effects of the three other major discharges from the estuaries of the Tyne, the Wear and the Humber.

The coastal area investigated extended from Druridge Bay in Northumberland to Donna Nook in Lincolnshire. The aim of the expanded programme was to examine the effects, if any, of the effluent-bearing waters from the four estuaries on the coastal waters of the North Sea. Hydrographic surveys were undertaken to examine the dilution and dispersion of estuarine water and hence predict the coastal areas where effects on the benthos were most likely. The hydrographic and benthic biological studies were supported by extensive monitoring of water quality, sediments and biota by chemical analysis.

The Results

Hydrographic Investigation

The investigations included detailed measurements of current velocities and the salinity and temperature throughout the water column. Tidal ranges and current velocities were similar off the Tyne, Wear and Tees but off the Humber estuary they were greater. Stratification of the relatively low salinity waters emanating from the Tyne, Wear and Tees hinders vertical mixing so that dispersion throughout the water column occurs some distance from the estuary mouth. The Wear is smaller and more stratified than the Tyne and Tees, and its dilution of effluents is comparatively low, but available dilution in the adjacent coastal waters is higher.

By comparison, the Humber estuary is very much larger than the other three estuaries and there is little stratification. Substantial mixing occurs within the estuary, but further dilution by the coastal waters is relatively slow.

On an ebb tide, the intertidal volume of an estuary is discharged into the open sea. The mean water depth in the coastal zones off the four estuaries was approximately 20 metres. Assuming this depth, the intertidal volumes were used to estimate the areas of spread of the ebb tide discharges. These areas are tabulated below :

Tyne	Wear	Tees	Humber
1.0	0.2	1.5	67.5 kilometres ²

Residual currents along the North East coast of England are around 1.5 cm/second. The residuals usually run either North or South, depending on wind direction and the barometric pressure gradient. Tidal movements promote mixing, but have little effect on the residual movement of contaminated water away from the vicinity of estuaries.

Dissolved Inorganic Nutrients and Heavy Metals

Water sampling was carried out along transects adjacent to the four estuaries and in a control area at Robin Hood Bay, Yorkshire. This control area was approximately midway along the length of coast under study and well away from all the four estuaries.

A total of 700 samples were analysed for nutrient and heavy metal. The results of the metal analysis showed that the concentrations of dissolved cadmium, copper, lead, manganese, mercury, nickel, silver, vanadium and zinc were all within a factor of three of the concentrations found in oceanic water. The level of cobalt was an order of magnitude higher. An increase in concentration of dissolved metals arising from discharges of contaminated water from Tyne, Tees and Humber was detected ; however, the increase was of the same order of magnitude as those arising from the presence of naturally occurring sea bed sediments rich in heavy metals. Such metalliferous sediments are found between Saltburn and Runswick Bay in Yorkshire.

The highest mean levels of dissolved inorganic nitrogen (as nitrate and ammonia) were found off the Tees and Humber estuaries. The concentration of dissolved phosphate was greater in the inshore waters North and South of the Humber than elsewhere in the coastal zone.

Stratification of nutrients through the water column was very much more marked in Teesbay than off the Humber, confirming the hydrographic observations in these areas. Seasonal changes in nutrient concentrations followed the expected pattern of low concentrations in summer and high values in winter. The nutrient levels found in Tees Bay between 1972

and 1975 were consistently greater than in areas further North, largely because of the discharge of untreated sewage and industrial wastes into the estuary.

Heavy Metal Concentrations in Sediments and Selected Benthic Species

A total of 224 sediment samples from the Tyne, Tees and Wear areas have been analysed for their heavy metal content. No sediments suitable for analysis were obtainable from the Humber area.

In comparison with an unpolluted area (Tor Bay in Devonshire), overall average concentrations of copper, manganese and zinc are 2 to 3 times greater off the North East coast of England. Concentrations of cadmium, chromium, cobalt, lead, mercury and nickel were similar to those in Tor Bay.

Within the study area, the distribution of concentration was not uniform. Sediments from the Tyne and Wear area and from between Saltburn and Runswick Bay had above-average concentrations of all metals. By contrast, mercury was the only metal present at above average levels close to the Tees estuary.

A total of 2,000 animals of four common species have been analysed for their heavy metals content. The four species were the starfish Asterias rubens, the edible crab Cancer pagurus and the molluscs Nucula turgida and Tellina tenuis. A wide range of metal concentrations were found in these animals but there does not appear to be any simple correlation with metal concentrations in seabed sediments, or in the levels of dissolved metals in the over-lying waters.

The Benthic Fauna

The population of benthic animals in soft sediments has been sampled in fifteen selected areas, extending from Druridge Bay to Hornsea in Yorkshire. A large number of samples have been taken with a Smith-McIntyre grab. Initially a "grid" of 20 positions was sampled in each area. Subsequently a series of ten replicate samples were taken from each of two positions in every area. Replicate sampling has permitted a statistical analysis of the data, and the large volume of information has been processed using computer techniques. The greatest diversity of species (221 individual species) occurs in the northern areas of Druridge Bay and Cambois Bay. Diversity is also high in the two southerly areas of Filey and Hornsea bays. Between these areas, in Tees Bay, and off Skinningrove and Boulby diversity is lower (167 individual species), but very large numbers of individuals were recorded for the common species.

Conclusion

It was envisaged at the start of the contract that a period of 3-4 years work on the enlarged survey area would be possible. Consequently, the first year (1974-75) of the study was designed to be a preliminary investigation of the enlarged area. This investigation was a data-gathering exercise, involving intensive sampling to obtain information on the hydrography, chemistry and biology of the area. These data would have been carefully assessed, and more detailed studies developed in subsequent years, as has been the case in Tees Bay. However, as the work was confined to one year, the benthic studies have not been fully developed. Several years study are necessary before any realistic assessment of the effect of polluted estuaries on the benthos of adjacent coastal areas is possible. Nevertheless, a large volume of information has been acquired, and this material, together with the hydrographic and chemical data, has been presented in the full report.

Publications

The Effects of Four Industrialised Estuaries upon the Coastal Waters of North Eastern England. Brixham Laboratory. BL/A/1698

Contractor: UNIVERSITY OF DURHAM
Contract No. O74-74-1 ENV. C.K.
Project leader: Dr David J. Bellamy
Title of project: Baseline studies of the pollution of inshore waters

Aim

To make use of various measurable attributes of Kelp Forest and related ecosystems to monitor the inshore marine environment with special reference to pollution.

"Core Study"

A "core study" of 35 sites dominated by Laminaria hyperborea covering the coasts of Britain (but concentrated in the North Sea) demonstrated the existence of the following biotic gradients which show correlation with known or suspected gradients of pollution.

- (1) A reduction of the depth range of the forest with increasing 'pollution'. Limitation by light was checked in each case by biomass studies.
- (2) A decrease in the species diversity of the holdfast infauna (only animals that exceeded 2 mm in any dimension were used) with increasing pollution.
- (3) An increase in the total number of individual animals in each holdfast sample in areas subject to pollution by (organic matter) sewage, in every case this was due to massive recruitment of suspension feeders.
- (4) An increase in the amount of one or more of the following heavy metals (Pb, Cu, Ni, Cd, Zn) in the stipe tissue of the kelp with increasing pollution.

SATELLITE STUDIES in unpolluted waters of Scotland confirmed,

- (1) that adequate sample sizes (3 litres of holdfast) were being used,
- (2) that any influence of freshwater can be ruled out as a 4% decrease in salinity effectively eradicates the kelp on

on which the monitoring system is based, (3) that the bedrock type (the study included serpentine) has no effect on any of the measured attributes, (4) that kelp plants that were exposed in situ to experimental "pollution" by organic matter (compost) or one of the following, Zn, Cu, Pb or Ni over a period of 50 days showed the following changes:- (a) all heavy metal treatments brought about a significant decrease in the number of species in the holdfast infauna; (b) significant increases in Cu and Zn were recorded in the stipes of the treated plants. No similar increases in the amounts of Pb and Ni were found and it is of interest that these metals had the least effect on faunistic diversity; (c) the "compost" treatment brought about a slight but not significant increase in the diversity of the infauna, this was not accompanied by an increase in the number of individuals present nor in the proportion of suspension feeders.

EXTENSION STUDIES along the coast of mainland Europe showed:-

(1) that kelp forest dominated by Laminaria ochrolevia shows similar characteristics and hence may be regarded as interchangeable for monitoring purposes. Direct comparison between the two types of kelp forest must however be treated with caution.

(2) The isolated deep water populations in the Straits of Messina are ecologically too different to allow direct comparisons with Atlantic coast populations to be useful. However they do provide a convenient point of reference linking monitoring networks in the two main marine areas that are adjacent to Europe.

IN RELATION TO THIS (3) comparative studies of the benthic communities on rock in the Mediterranean showed similar faunistic changes, viz. reduced diversity and an increased abundance of suspension feeders passing into the polluted waters of the Bay of Naples.

(4) A satellite base line study of 31 sites around the Tyrhenian Sea employing Arbacia lixula and Paracentrotus lividus (both of which are associated with the Straits of Messina reference site) to monitor the levels of heavy metals has been established.

Publications (In Press)

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Sheppard C. R. C. (1977) Some physiological and ecological gradients around Naples. Proc. Conf. on Marine Parks at Castellabate.

IN PREPARATION

Sheppard C. R. C. The holdfast fauna of Laminaria hyperborea (Gunn.) Fosl. measurements of samples and minimal sample size.

Sheppard C. R. C., Bellamy D. J. & Sheppard A. L. S. Responses of the kelp holdfast ecosystem to various natural and man made (pollution) gradients.

Pfister A. A critique of the use of the Pressure and Microwave digestion techniques for comparative studies of heavy metals in marine organisms.

Pfister A. A baseline study of heavy metals in the inshore environment of the Tyrhenian Sea.

Contractor: UNIVERSITY OF DURHAM, ENGLAND (DEPARTMENT OF BOTANY)
 Contract No: 074-74-1 ENV UK (Project 2)
 Project leader: Dr B. A. Whitton
 Title of project: Effects of lead and zinc pollution on vegetation in
 flowing waters

ABSTRACT

Data have been collected concerning 200 10 m reaches of fast-flowing waters enriched by zinc and/or lead as a result of mining activities. These data are stored on computer disc, together with similar data for unpolluted streams. The data include composition of sediment and water, semi-quantitative account of the flora, metal composition of certain submerged plants and laboratory assays of the tolerance to zinc of filamentous green algae. Some results (especially those dealing with Zn) have been published; further papers are in preparation.

LOCATION OF MAIN REGIONS SURVEYED

Belgium	R. Gueule and tributaries
France	Brittany; R. Lot and its tributary, Rieu Mort
Germany	Aachen region; Harz; R. Sülz
Ireland	R. Avoca and tributaries; Silvermines
Italy	Sardinia: various sites, especially F. Flumendosa and Iglésias regions
Netherlands	R. Geul
U.K.	mostly N. England (Alston Moor Orefield); also Fife (Scotland) and N.W. and W. Wales.

RESULTS

1. The occurrence of Zn, Cd and Pb as a result of mining activities is strongly inter-correlated. It is often difficult to separate the effects of one element from effects due to the others.
2. For ecological purposes, most flowing waters with high levels of zinc as a result of mining fall into the following types:
 - (i) Highly acidic drainages, with $\text{pH} \leq 3.0$, and most heavy metals in solution. (In this account, metal passing through a 0.2 μm Nuclepore filter is termed dissolved.)
 - (ii) Waters with conspicuous deposition of silt.
 - (iii) Waters with $\text{pH} \geq 6.0$ and lacking conspicuous silt.

3. All flowing waters of (i) and almost all of (iii) have at least some photosynthetic organisms. The only exceptions are a few streams in Sardinia where flow is not permanent. In contrast, waters of (ii) are sometimes devoid of any photosynthetic organism (Rieu Mort, F. Flumendosa, main drainage stream at Iglésias) or almost so (R. Avoca).

4. The highest levels of dissolved metals were all found in Sardinia (Zn, 2670 mg l⁻¹; Cd, 10.0 mg l⁻¹; Pb, 3.1 mg l⁻¹). The most extensive areas with streams and rivers enriched by Zn, Cd and Pb are probably the Harz and the Alston Moor Orefield.

5. The specialized flora of acid drainages with high zinc is similar to that of other acid drainages, whether with other heavy metals or with low levels of any heavy metal (Yellowstone, U.S.A.). The characteristic species at higher pH values and with very high zinc levels may be summarized:

- (i) calcareous, no sewage: *Plectonema gracillimum*, *Mougeotia*, *Achnanthes minutissima*, *Dicranella varia*, *Agrostis stolonifera*.
- (ii) non-calcareous, no sewage: *Hormidium* spp., *Dicranella* spp.
- (iii) with sewage effluent: *Stigeoclonium tenue*, *Leptodictyum riparium*.

6. The flora of waters with moderate levels of dissolved Zn (0.4 - 1.0 mg l⁻¹) is usually quite similar to that of adjacent waters with lower Zn. Nevertheless transplant experiments suggest that many of the algae present in the former are in fact adapted to the presence of the zinc.

7. The filamentous algae *Hormidium rivulare*, *H. flaccidum*, *H. fluitans* and *Stigeoclonium tenue* all occur over a wide range of Zn levels. However the genetic tolerance of a population closely reflects the Zn level at the site. The threshold level above which adaptation occurs is influenced by other factors, but it is probably sometimes as low as 0.2 mg l⁻¹ Zn.

8. Both field and laboratory studies indicate that the main factors reducing Zn toxicity may be summarized as follows:

	<i>H. rivulare</i>		<i>Stigeoclonium tenue</i>		
	populations	sensitive	resistant	sensitive	resistant
Ca		+++	++	+++	+++
Mg		++	+++	++	+++
P		+++	+++	0	+

In general the higher the pH, the more toxic the Zn, provided that it is still in solution. The other key factors increasing toxicity are Cd and Pb. There is a marked synergistic interaction between the toxicities of Zn and Cd to *Hormidium*.

9. A clear relationship exists between the levels of Zn, Cd and Pb in some plants (e.g. *Lemanea*) and those in the water. Zn in *Lemanea* is more sensitive to short-term changes than Cd and Pb, and a marked change in environmental Zn could be detected within one hour. No marked differences in the kinetics of Zn exchange were observed between plants from polluted reaches and ones from non-polluted reaches when transferred to high Zn levels. If genetic adaptation to Zn exists in *Lemanea*, it does not affect its use for monitoring Zn.

LIAISON WITH OTHER RESEARCH GROUPS

The project has led to close liaison with several other research groups, especially Institut für Allgemeine Botanik und Botanischer Garten, Universität Hamburg (Prof. A. Weber) and Laboratoire d'Hydrobiologie, Université Paul Sabatier, Toulouse (Dr H. Decamps).

CONCLUSIONS

The floras of waters with high levels of zinc are very reduced, but standing crops are sometimes high if deposition of silt is slight. The combination of high zinc and sediment sometimes leads to the absence of any photosynthetic organism. With moderate zinc pollution it is often difficult to detect floristic differences from unpolluted streams. Nevertheless many algae in the former are adapted to the presence of the zinc. In those species investigated critically, adaptation is genetic. It seems probable that adaptation sometimes occurs at levels of 0.2 mg l^{-1} Zn, or even lower. Such levels are little if any higher than those occurring in the rain near some industrialized regions.

Other factors have such marked effects on the toxicity and accumulation of Zn, Cd and Pb, that it is not possible to predict the effects of a particular level of a metal without additional environmental information. For instance, the synergistic interaction between zinc and cadmium to *Hormidium* is so marked, that interpretation of the data about one metal is of limited value without data about the other. Although flowing waters with high zinc levels have characteristic floras it is suggested that assays of tolerance and metal composition are likely to prove of most use for 'monitoring' aquatic environments. The probability that genetic adaptation for tolerance of a heavy metal is widespread means that it is important to know the behaviour of a particular species before using it to monitor metal levels. In particular, organisms should not be used as laboratory monitors without knowledge of the field environment from which they were taken, and of the likelihood of subsequent adaptation taking place in the laboratory.

PUBLICATIONS (acknowledging contract)

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Contractor : Field Studies Council, London
 Contract n° 076-74-1 ENV UK
 Project Leader : J.M. BAKER
 Title of project : 1) Milford Haven sublittoral survey
 2) Celtic Sea macrobenthos survey
 3) Gas Liquid Chromatography analyses.

SUMMARY REPORT OF WORK CARRIED OUT FOR THE E.E.C.

BY THE FIELD STUDIES COUNCIL, OIL POLLUTION RESEARCH UNIT.

The work funded by the E.E.C. falls into three categories:

- 1) Milford Haven sublittoral survey;
- 2) Celtic Sea macrobenthos survey;
- 3) Gas Liquid Chromatography analyses.

1) Milford Haven sublittoral survey.

J. M. Addy

I. Grab-sampling in 1974 and 1975.

Until recently no quantitative work had been done on the soft sublittoral deposits of Milford Haven. A preliminary sublittoral survey was carried out in 1974, and this survey was repeated with more intensive sampling in May 1975.

The aim of this work is to extend the range of the Oil Pollution Research Unit's monitoring programme. It was thought that the design of a suitable monitoring programme could best be achieved with reference to the overall arrangement of benthic macrofaunal communities in Milford Haven.

Thus, the first stage in setting up some form of monitoring programme was an investigation of the spatial and numerical distribution of macrofaunal association throughout the survey area.

Methods.

A Day Grab designed to sample an area of $0.1m^2$ to a depth of about 10 cms was used in 1974 and 1975.

All the sediment was subjected to an initial screening on board the vessel, using a 1mm sieve and a sea water hose. Everything retained on the 1mm mesh was stained with eosin and preserved in 5% formalin in seawater.

After sorting into the main groups, specific identification was made where possible.

Results.

These surveys have indicated a wide variety of sediments and associated communities throughout the area, though most stations had a fauna which approximated to the Abra alba communities described from other areas of shallow waters with mixed muddy sand bottoms.

Abra alba was found in greatest numbers in the mouth of Milford Haven, though the numbers tailed off in the finer sediments to the east and the coarser sediment of the outermost stations.

It has been possible to produce an ordination in the data, reflecting the gradient in sediment types from clean sand and gravel in the mouth of the Haven to fine stable muds in Angle Bay.

It is clear that the occurrence of Abra alba is limited at both ends of this ordination, and the performance of the Abra community at the limits of its distribution is of particular interest.

II. Shallow water sampling by diving - 1977.

Investigations of the sublittoral macrobenthos have moved from the broad surveys of 1974 and 1975 to more detailed work around specific pollution sources in Milford Haven.

A grid of sampling stations around a refinery effluent were visited in summer 1977 using a diver-operated suction sampler. These samples have not yet been sorted.

In addition to biological sampling, measurement of a number of hydrographic parameters was carried out, and it is hoped to use gas chromatographic techniques to evaluate the oil content of sediments throughout the grid of sample stations. This work is continuing.

2) Survey of the Celtic Sea macrobenthos.

J. P. Hartley

The Celtic Sea is an area of oil industry interest in which the benthic (sea bed) fauna was poorly known. In an attempt to rectify this situation, before oil exploration began in earnest, a survey of the sublittoral macrobenthos (i.e. animals greater than 1mm) was carried out during the summers of 1974 and 1975.

A total of 83 stations were sampled, using a variety of gear from the research vessels "Challenger", "Prince Madog", "Corella" and "Kay BB". The locations of the sampling sites and their relation to blocks licensed for oil exploration are shown in the figure.

At most sites, separate samples of the epifauna and the infauna were taken. The samples were preserved on board and returned to the laboratories. Samples of the sediment were also taken for particle size analysis.

The samples were analysed in the laboratories and the organisms present were identified as far as possible. A type collection of all species found was made for reference purposes.

A-large number of species was recorded, the final total being in excess of 700 taxa. The information obtained on the distribution and associations of the Celtic Sea benthos is of considerable scientific value, especially from the zoo-geographical point of view. A number of species have been added to the British fauna, and the ranges of several forms found in British waters have been significantly increased.

The survey should not be regarded as a 'baseline' for any future pollution monitoring. Rather, it should be viewed as a framework of physical and biological data for future use in identifying areas of particular scientific or environmental value.

3) In addition to the Milford Haven and Celtic Sea biological surveys, a gas liquid chromatography unit was purchased and is now being operated by an OPRU chemist. This GLC unit is used in conjunction with an ultra-violet spectrophotometer for the analysis of sediment hydrocarbon levels and the initial 'fingerprinting' of crude oils for correlation with the sediment data.

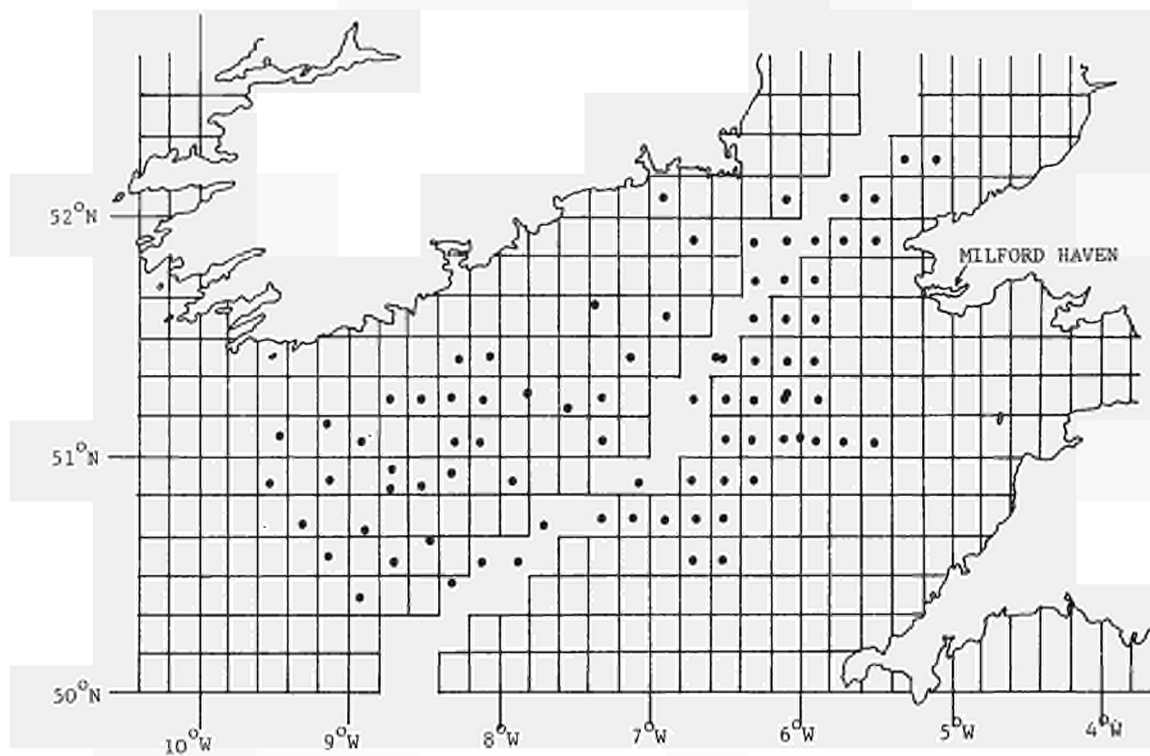
Several other related projects have been/are carried out by OPRU, mainly financed from other sources. The areas intensively sampled for monitoring purposes are briefly described below, together with further details of the Celtic Sea and Milford Haven surveys.

<u>Area Surveyed</u>	<u>Year</u>	<u>Number of Sites</u>	<u>Number 1/10m² Replicates</u>
Celtic Sea	1974 & 1975	83	N/A
Milford Haven	1974	25	5 + 10
Milford Haven	1975	25	10
Southampton Water	1975	29	10
Southampton Water	1976	30	10
Ekofisk Oil Field	1973	24	10
Ekofisk Oil Field	1975	24	10
Ekofisk Oil Field	1977	24	10
Forties Oil Field	1975	19	5
Auk Oil Field	1977	12	5
Sullom Voe	1976	30	10
Sullom Voe	1977	20	10
Moray Firth	1977	28	5 + 10

Publications.

Addy, J. M., 1976. Preliminary investigations of the Sublittoral Macrobenthos of Milford Haven. In "Marine Ecology and Oil Pollution". J. M. Baker, Ed., Inst. Petr.

Locations of the Celtic Sea sampling sites.





Map of Milford Haven showing Grab sampling stations and areas at which Abra alba occurs in high densities (> 50 per m^2).

Contractor: University of Cambridge
Contract no.: O79-74-1ENV UK
Project Leader: S.M. Haslam
Title of Project: River vegetation and pollution

Flow regime, rock type and the influence of man are the most important factors affecting river vegetation. The ecology of river plants in Britain has been investigated in order to understand this ecosystem, and so to be able to use macrophytes to assess habitat factors, including pollution and other effects of man.

The principle effects of flow regime and rock type were studied in earlier, non-C.E.C., contracts. The research during this contract concentrated on the (natural) nutrient status of plant communities (as a pre-requisite for understanding pollution), the distribution of the effects of pollution and of possible pollutants, the effects of management factors, and the study of river ecology.

Water plants obtain nutrients from both water and sediment but, because the sediment contains the most, uptake is mainly from there. (As most pollutants enter streams as solutes, uptake from water could be more important in pollution toxicity). Most nutrients occur in silt and mud (hereafter jointly termed silt), and so the proportion of silt in the substrate, and the amount accumulated on plants, are important for nutrition.

Some 650 silt samples from throughout Britain were analysed for the 9 major nutrients and 14 heavy metals. About 200 samples were analysed for anionic detergents, tannins and lignins, CN, CO₂, alkalinity and volatile acids, and a smaller number for organic carbon, relative stability, dissolved O₂, phenol and oil-in-water.

The differences in vegetation between streams on different rock types is due to differences in topography (affecting flow regime), silting (affecting the amount of silt - nutrient-containing material - in each habitat) and in the concentrations and proportions of available nutrients in that silt. The main British rock types (soft limestone, soft sandstone, clay, hard limestone, hard sandstone, Resistant rocks, Coal Measures, silt alluvium

is either static or improving. Canal surveys showed, contrary to expectation, that only one (out of 6) had deteriorated, despite the increase of pleasure boats between 1972 and 1976.

The general ecology of streams, the effects of management, such as dredging, cutting, herbicides, regulation of flow, and the associated effects of boats, trampling, grazing etc. are, with the principles of predicting their effects, described in "River Plants" (see below).

PUBLICATIONS

Haslam, S.M. (1978) River Plants. University Press, Cambridge.

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and peat alluvium) can be distinguished and characterised for vegetation by these factors.

Many substances cause pollution. In most British streams whose vegetation is altered by pollution, however, the cause is town effluents, i.e. sewage and industrial effluents. Species diversity is decreased, species balance is altered (some species being more tolerant than others), vegetation quantity is decreased, and Potamogeton pectinatus is often increased. The nutrients, heavy metals, and other pollutants tested are not, either singly or in combination, responsible for the main toxicity, though the absence of plants by the outfalls of bad-quality effluents could be due to combinations of pollutants - chemicals in concentrations which are harmless individually can be lethal when occurring together. The active agent in town pollution is not yet known, but is thought to be organic, acting through the sediment as well as, or more than, through the water. It is thought to act by lessening root development (decreasing root length, density and branching) so that plants anchor less well, and are more easily washed away. Under severer pollution shoots may become lush and flaccid also, and in yet worse habitats shoot development is hindered as well.

Vegetation is also affected by other pollutants. Eutrophication (fertiliser, some town effluents) leads to a more eutrophic but natural plant community. Salt-pan effluents brings in luxuriant Potamogeton pectinatus. Coal mine sediment effluent leads to unstable substrates in hill streams and so to lessened total vegetation though perhaps the retention of Potamogeton pectinatus (brought in by the associated town effluents). Herbicides are deliberately used for weed control in alluvial dykes, but they and pesticides also cause damage through used containers etc. being dropped into watercourses and (at least in N. America) being washed off the land.

An 8-point pollution index is being developed. This assesses the chemical damage to the vegetation. It is designed particularly to diagnose town pollution and eutrophication, but when species tolerant and sensitive to other forms of pollution are known, the index can be modified to assess these.

Repeat surveys of British watercourses show that, for river plants, pollution is only occasionally getting worse. In general, river vegetation

Contractor: Natural Environment Research Council

Contract No: O85-74-7 ENV UK

Project leader: Dr B L Bayne

Title of project: The ecological effects of sub-lethal stress in estuarine invertebrates.

The main objective of this study was to establish whether different populations of mussels (Mytilus edulis L.) could be distinguished physiologically, and to relate any such differences to environmental conditions that are known, from laboratory experiments, to have physiological effects on these animals. Measurements were made on mussels from six sites, chosen to represent different ecological conditions. Regular visits to these sites were made using a mini-bus equipped as a mobile laboratory. On each visit the following physiological variables were measured under conditions appropriate to the local population; rates of oxygen consumption, feeding and ammonia excretion, assimilation efficiency, and the gametogenic stage. The results were analysed by regression and covariance techniques and are presented, where appropriate, as weight-corrected values typical of the animals at each site.

At all sites oxygen consumption varied seasonally, with high values occurring when the animals had well-developed gonads. Rates of feeding were less variable seasonally and were lower than had been predicted from laboratory data, possibly as a result of high particulate loads at most of the sites. Average values were as follows (for mussels of 1 gram dry flesh weight):

Lynher	1.67 ± 0.26	1 h^{-1}
Cattewater	1.84 ± 0.18	"
Mumbles	0.73 ± 0.13	"
Kings Dock	1.58 ± 0.36	"
Swale	0.72 ± 0.21	"
Roscoff	1.25 ± 0.30	"

Rates of ammonia excretion varied seasonally, with high values in the summer, low values in the spring and winter. The assimilation efficiencies for particulate material cleared from suspension were low:

Lynher	20.6 \pm 8.3%
Cattewater	27.5 \pm 11.7%
Mumbles	20.4 \pm 4.3%
Kings Dock	34.5 \pm 9.4%
Swale	30.8 \pm 5.1%
Roscoff	27.3 \pm 5.7%

The physiological condition of mussels at each site was assessed in terms of two stress indices, the scope for growth (an index of energy available for growth and the production of gametes) and the oxygen: nitrogen, or O:N, ratio (an index of protein catabolism). The scope for growth varies seasonally from low values, often negative, in the winter to maximum values in the summer (Fig. 1). The O:N ratio takes minimum values in the summer after the mussels have spawned, with maximum values in the winter.

Population differences were seen in both these indices. Ranking populations from high values (= good condition) to low values for both indices were similar:

<u>Scope</u>	<u>O:N</u>
Lynher	Lynher
Cattewater	Cattewater
Roscoff	Roscoff
Mumbles	Swale
Kings Dock	Kings Dock
Swale	Mumbles

Of these 6 populations, Lynher and Roscoff were, by general assessment, the least polluted; Kings Dock and Swale were most polluted. At Mumbles the mussels are subjected to greater wave exposure than at other sites and there is also some local sewage pollution. The Cattewater population was originally chosen to represent mussels exposed to thermal effluent, but over the period of these measurements the local power station was on minimal supply and the water temperatures were seldom higher than normal

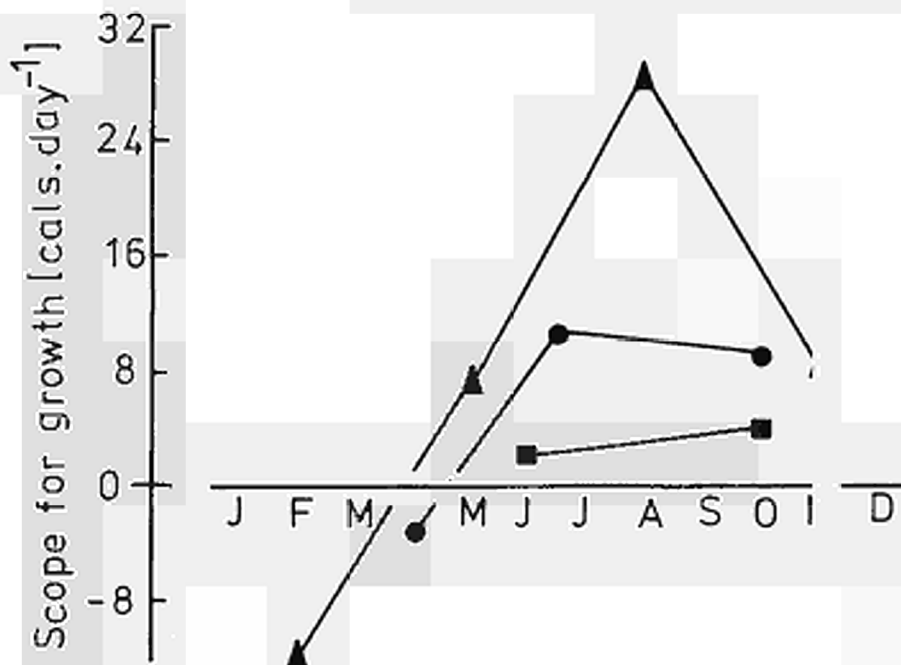
ambient values in the neighbouring estuary.

The study demonstrated that it is possible to recognise differences between populations as based on physiological criteria. It also served to demonstrate the feasibility of carrying out certain physiological measurements in the field under natural conditions. The data suggest that continued studies of this kind will prove useful in making assessments of the impact of both natural and man-induced alterations in estuarine environments.

Publications

1. B.L. Bayne, J.Widdows and C. Worrall, 1977.
"Some temperature relationships in the physiology of two ecologically distinct bivalve populations". In: Physiological responses of marine biota to pollutants, eds. V. Vernberg, A. Calabrese, F. Thurberg and W. Vernberg. Academic Press, 379-400.
2. B.L. Bayne and J. Widdows, In preparation.
"The ecophysiology of two populations of Mytilus edulis L."

Fig. 1. The scope for growth (calories per g) in three populations of *Mytilus edulis*:
 ▲, Lynher; ●, Mumbles; ■, Kings Dock.



Contractor: Natural Environment Research Council
Institute of Marine Biochemistry
Aberdeen, Scotland

Contract No. 086-74-7 ENV. U.K.

Project Leader: Dr Thomas L. Coombs

Title of project: Metabolism and Mechanisms of Heavy Metals, including lead by Estuarine Invertebrates.

Introduction:

This study of the mechanisms of uptake and storage of lead by estuarine and freshwater molluscs was undertaken firstly to establish the basic metabolic patterns for the assimilation of this element by shellfish and secondly to explore the effects that natural complexing agents might exert on the system. The study of lead forms part of an overall research programme of The Institute of Marine Biochemistry, which is concerned with the uptake, storage and elimination of metals in marine and freshwater organisms.

Problems in trace metal analysis at nanogram/ml concentration levels and the replacement of the technical assistant at a crucial point in the investigations have prevented completion of the project as originally described. This has resulted in the postponement of the studies on the freshwater mussel, Anodonta, which could well form the basis of a future project. Significant changes have been observed using the estuarine mussel, Mytilus edulis, however, and these are described briefly below.

Materials and Methods:

Mussels for use in experimental tanks were collected in batches of several hundreds from the Ythan estuary (an estuary some twenty kilometers from Aberdeen and thought to be free of industrial pollution). The mussels were mature specimens, 6-7cm shell length, and acclimated in the laboratory sea water aquarium for at least five days, before being exposed to lead. Batches of ten-twenty were kept, unfed, in four litres aerated seawater at 15°C contained in separate polyethylene containers, into which known concentrations of lead in different defined complexes had been added. In one experiment, the seawater was first filtered through a 0.45µ Millipore cellulose filter to remove particulate material. The 4l volumes of seawater (± lead) were each replaced every second day. Four specimens from each container were removed at known time intervals and dissected into gills, mantle + gonads, visceral mass (gut + diverticulum), foot, adductor muscles and kidney, except for the initial high lead concentration studies, where the kidney was included together with the gills. The separated tissues from each animal were then combined and after determination of the wet and dry tissue weights, were either wet-ashed with concentrated nitric acid in sealed Teflon bombs for 30 mins at 110°C, or dry ashed in platinum dishes at 500°C in a silica-lined muffle furnace and the ash dissolved in dilute hydrochloric acid. The acid solutions were

then analysed by atomic absorption spectroscopy for zinc, copper, iron and manganese and by anodic stripping voltammetry for lead and cadmium. Selected animals were also dissected separately and the tissues fixed in buffered glutaraldehyde for subsequent sectioning and examination in the electron microscope. For the field study, samples of mussels and estuary water were taken at monthly intervals from The Ythan estuary (unpolluted) and The Dee (Aberdeen Harbour, polluted). The mussels were treated as described above, while the water was immediately filtered through a 0.45 μ Millipore cellulose filter (acid washed prior to use) and the filtrate collected in an acid washed polyethylene bottle and 5ml 1N Hydrochloric acid (Aristar, trace-metal-free) added per litre of filtrate. The filtrates were stored frozen at -15°C together with the sediments collected on the Millipore filters for subsequent analysis.

Lead Uptake and Tissue Distribution:

The initial experiments were designed to stress the animals with a high concentration of lead, in order to maximise any changes that might occur. Mussels were exposed to 5 μ g Pb/ml, added as lead nitrate, for ten days. The use of filtered versus unfiltered seawater allowed any contribution from absorption or adsorption of the lead onto particulate matter to be assessed.

There was uptake of lead in every tissue, with the gills + kidney showing a very high concentration (20,000 μ g/g dry wt., concentration factor = 4,000) after ten days exposure. This concentration in the gills was some five-fold higher than in the visceral mass, ten-fold higher than in mantle and between ten and twenty-fold higher than in foot and adductor muscles. The very high concentration in the gills is similar to the concentrations found in whole soft tissues of mussels by Schulz-Baldes, 1974, after exposure to the same concentration of lead.

When the lead concentration in the seawater was reduced to 0.1 μ g Pb/ml in later experiments, a similar tissue distribution was found with the highest concentration occurring in the isolated kidney, (300 μ g Pb/g dry wt. tissue, concentration factor = 3,000). This was some thirty-fold higher than the concentrations found in the gills and visceral mass.

There was no marked difference in the rates or the extent of lead uptake between filtered and unfiltered seawaters, implying that the presence of particulate material is not a major factor in influencing the uptake of this metal.

Histochemistry:

The electron microscope studies revealed high concentrations of electron dense particles in the gills and in the kidney. An X-ray Microprobe analysis of these particles was carried out by courtesy of Associated Electrical Industries Ltd., Manchester, England using a

CORA analytical electron microscope. The analysis confirmed that the small extremely dense particles in the cytoplasm of the gills and the kidney were lead deposits as well as the amorphous greyish material seen within pinocytotic vesicles in the gills, digestive diverticulum and gut. The lead found in these membrane bound vesicles was associated with some other material, probably mucus. For the kidney, however, there were indications that the large dark aggregates of lead are not membrane bound, with, in some instances, clear white spaces next to the aggregates occurring. These are thought to appear as a result of volatilization of some of the lead during exposure to the electron beam, a further indication perhaps of the unbound nature of the kidney deposits. No evidence could be found of any intranuclear inclusion bodies, as has been described by Goyer *et al.*, 1970 in rat kidneys after exposure to lead.

Thus the uptake of lead in the absence of any added complexing agents appears to be mediated by transport in membrane bound vesicles and associated with some non-metallic high molecular weight material. This mechanism seems to be very similar to the situation found for the uptake of iron, present as particulate hydrated ferric oxides, by mussels, George, Pirie and Coombs 1975.

The electron microscope studies of the tissues, which have been exposed to lead in the presence of complexing agents, showed the presence of lead-containing, membrane-bound vesicles similar to those seen with lead nitrate.

Effect of Lead on The Naturally Occurring Trace Metals:

It is of importance to examine the effect of lead on the other metals present, since, in addition to any direct toxic effect, a metal can induce an indirect metabolic effect either by competing with the other essential trace metals, producing a conditioned deficiency, or by stimulating an enhancement of the other metals, resulting in an excess or remobilization of these metals (synergisms). This aspect was examined for zinc, copper, iron, manganese and cadmium found in the Ythan mussels. In general there is a tendency for the other metal concentrations to increase. This was found to be particularly noticeable for manganese and copper in the mantle at both 0.1 and 5µg Pb/ml concentration and the viscera at 0.1µg Pb/ml only. The zinc concentrations, on the other hand were found to be remarkably constant. The cadmium analyses proved to be too variable to be able to deduce that any significant trends were occurring. The changes that have occurred in zinc, copper, iron and manganese are summarised in the table on the following page:

	Gills		Mantle		Kidney	Viscera		Muscle		Foot	
	(a)	(b)	(a)	(b)	(a)	(a)	(b)	(a)	(b)	(a)	(b)
Zinc	0	↑	0	0	0	0	↑	0	↑	0	0
Copper	0	↑	↑	↑	0	↑	0	0	0	0	0
Iron	0	0	↑	↑	0	↑	0	↑	0	0	↑
Manganese	0	↑	↑	↑	↓	↑	0	0	↑	0	0

↑ = increase

↓ = decrease

0 = no significant change

(a) = 0.1µg Pb/ml

(b) = 5.0µg Pb/ml

These effects by lead on the other trace metals are in contrast to the action of cadmium on the metals in mammalian and plaice, Pleuronectes liver and kidney tissue, (Bremner, 1974, Coombs, 1974), where there are significant decreases in copper and zinc.

The metabolic and enzymic consequences of an increase in copper, iron or manganese in Mytilus mantle or gills have not been explored.

The Influence of Complexing Agents on Lead Uptake

The high molecular weight compounds, humic acid, alginic acid, (main constituent of brown seaweeds), and pectin were chosen as representing naturally occurring complexing agents. These were compared to citrate as representing a small molecular weight compound. The high molecular weight complexes were prepared by dialysing a solution of each compound, buffered to pH 8.0, against several changes of a ten-fold volume of 0.1µg Pb/ml buffered solution with the lead present as lead nitrate. A portion of each lead-complex was then added to seawater to give a final concentration of 0.1µg/ml with respect to lead, i.e. the same as a control using lead nitrate. The uptake of lead was followed over a period of thirteen days, changing the lead containing seawater solutions each day. Representative samples of mussels were removed after three, six, ten and thirteen days exposure, dissected and either analysed for metals or fixed in buffered glutaraldehyde for electron microscopic examination as described previously.

There have been significant increases in the lead concentrations in all of the tissues for all of the lead-complexes, when compared to the concentrations obtained with lead nitrate. The largest increases were shown with citrate:- Adductor muscle and foot showing an eight-fold increase and gills, mantle, kidney and visceral mass a three to four-fold increase in the tissue lead concentrations. Humic acid, alginic acid and pectin on the other hand were equally effective in increasing the lead

concentrations only some two to three-fold. It is very probable that the lead is complexed to either hydroxyl or carboxylate-oxygen atoms in each of these complexes, the data above, therefore suggest molecular size may play an important role in determining the degree of enhancement of lead uptake. Further experiments using different complexing ligands as well as more well-defined complexes of graded molecular size are suggested. A potential lead toxicity hazard to higher member consumers in the shellfish food chains is indicated, where such naturally occurring complexing agents are present in relatively small amounts.

Field Studies:

A limited field study was undertaken in order to assess the situation in a natural habitat versus a polluted habitat. Unfortunately the preliminary water tissue analyses indicate the Dee estuary is not polluted enough in terms of lead and cadmium to produce any significant differences between the Dee and the Ythan mussel populations. The Ythan population, however, accumulates iron to a much higher concentration than that from the Dee, particularly in the gills, mantle and visceral mass, for example, Ythan mussel gills, 500 μ g Fe/g dry wt. Dee mussel gills, 300 μ g Fe/g dry wt. This difference may be a reflection of the muddy environment enjoyed by the Ythan mussels in comparison with a more rocky habitat for the mussels at the mouth of the Dee.

Summary:

Mytilus edulis have been exposed to lead in seawater at 0.1 and 5 μ g Pb/ml concentrations alone and in the presence of the naturally occurring complexing agents, humic acid, alginic acid, pectin and citrate. The mussels concentrated lead in all of the tissues without any obvious signs of distress, with the highest concentration being found in the kidney (3,000 fold concentration factor). Particulates in the seawater had no significant effect on the degree of lead uptake, but all of the complexing agents produced a marked increase in the tissue concentrations. Electron microscopic examination of the fixed tissues suggested uptake was mediated by pinocytosis of the lead together with some non-metallic high molecular weight material into membrane limited vesicles for transport, while storage in the kidney occurred as non-membrane bound aggregates. Uptake of lead additionally produced increases in manganese and copper concentration in the mantle and visceral mass, while zinc and iron concentrations were not greatly changed. A limited field study failed to detect any increase in lead in mussels from a local estuary polluted by industrial and domestic raw sewage inputs.

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Contractor: The Natural Environment Research Council, Alhambra
House, 27-33 Charing Cross Road, London, WC2H 0AX.

Contract no.: 087-74-7 ENV UK

Project Leader: Dr P. A. Plack

Title of project: Serum and egg lipoproteins in marine and freshwater fish, with specific reference to transport mechanisms for the entrainment in vivo of lipid-soluble pesticides and pollutants into eggs.

Normal serum lipoproteins and egg lipoprotein, found in both the eggs and the serum of female fish with maturing ovaries, were isolated from cod (Gadus morhua L.), a marine fish caught locally, and from rainbow trout (Salmo gairdneri Richardson), a freshwater fish supplied by local trout farms.

Egg lipoproteins prepared from the serum and from the eggs of cod have different molecular weights but similar proportions of lipid to protein, similar proportions of phospholipid, triglyceride and total cholesterol in the lipid, and similar amino acid compositions for the protein. A number of sub-units were found and a molecular rearrangement probably occurs during deposition in the ovaries. Egg lipoprotein and normal serum lipoproteins contain immunologically distinct sets of polypeptides, although the amino acid composition of the lipoprotein from cod eggs was broadly similar to that of apo-HDL-IV, the major apoprotein of cod HDL.

(¹⁴C)DDT added to trout serum in vitro was distributed within 5 min amongst the lipoproteins, including egg lipoprotein. When the DDT was added attached to one particular lipoprotein, it was rapidly distributed amongst all the lipoproteins. In each case the relative equilibrium concentrations per g lipid were VLDL 100, LDL 76, HDL 45, egg lipoprotein 46. A similar distribution was found after feeding DDT to trout. Although VLDL and LDL held more DDT per g lipoprotein lipid, HDL as the predominant lipoprotein in trout serum carried the major part of the DDT.

(¹⁴C) DDT fed to cod was rapidly distributed amongst the tissues, the relative concentrations per g lipid being liver 100, serum 44.7, muscle 18.8, brain 5.9. Similar relative values were found from 1 day to 14 days after feeding, and imply a rapid physical distribution rather than a distribution dependent on biochemical turnover of lipid.

(¹⁴C)DDT fed to trout did not show such a marked distribution pattern as with cod, possibly because of the more even distribution of lipid

stores and of the rapid metabolism of DDT and the inclusion of metabolic products in counts of radioactivity.

These results suggest that DDT may be attached to lipid molecules of a particular class or to a particular fatty acid. Analysis of cod tissues for phospholipid, triglyceride and total cholesterol indicated that these were not the classes of lipid involved.

Trout eggs do not take up egg lipoprotein to the exclusion of other serum lipoproteins. The normal serum lipoproteins, normal serum proteins and even bovine plasma albumin are also taken up. The uptake of egg lipoprotein or other serum lipoprotein is not essential for the uptake of DDT by trout eggs, but uptake of DDT was a time-dependent process.

Preliminary results with PCB's fed to cod showed that their distribution amongst the lipids of cod tissues differed from that of DDT.

Publications:

Mitchell, A. I., Plack, P. A. & Thomson, I. M. (1978). Relative concentrations of (^{14}C)DDT and of two polychlorinated biphenyls in the lipids of cod tissues after a single oral dose. Arch. Environ. Contam. Toxicol. 6, 1-8.

Plack, P. A., Skinner, E. R., Rogie, A. & Mitchell, A. I.
Distribution of DDT between the lipoproteins of trout serum.
(In preparation).

Contractors : The Natural Environment Research Council,
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Contracts No. 088.74. 7 Env. U.K.
082.74. 7 Env. F.

Project Leaders : A.D. Ansell,
H. Massé

Title of Project : Comparisons of the effect on benthic invertebrates
from the Mediterranean and from northern European
waters of temperature changes associated with
thermal pollution.

INTRODUCTION

The aim of this programme was to study, by a series of experimental laboratory observations, the effects on selected invertebrate species, of the types of thermal change usually associated with the discharge of cooling water from electricity generating stations in tidal waters. Under two contracts, studies have been made of species from inshore sandy sediments from the west coast of Scotland and from the French Mediterranean coast, an important aspect of the work carried out under the two contracts being a collaboration to examine how far geographical considerations affect the conclusions reached from experimental studies on the possible effects on the invertebrate fauna of discharges of heated water into the coastal zone.

The programme of studies proposed initially was designed to establish, 1) the ecophysiological basis of observed population changes in certain intertidal and subtidal invertebrates in areas subject to heated water discharge, 2) the sublethal effects of temperature changes especially in relation to factors affecting distribution and productivity of the benthos, and 3) the upper lethal limits of

temperature fluctuations tolerated by individual species. Revision of this initial programme within the limited time available under the 1st phase of the Environmental Research Programme resulted in an emphasis on the determination of upper lethal limits, although it has proved possible to design a protocol for these determinations which also provides valuable data on the sublethal effects of temperature on the burrowing response of many of the species examined. This response is of particular importance for those species, e.g., the bivalve molluscs, which normally live buried in the substratum relying on the burrowing response to maintain their position in the face of changes in profile caused by wave action. Observations were made on two main groups of animals; the bivalve and gastropod molluscs, from the macrobenthos of inshore sandy deposits, and the harpacticoid copepods from the meiobenthos of similar areas. In addition, a limited number of preliminary observations on other sublethal effect of temperature change were made, notably on aspects of the reproduction of certain gastropod molluscs, but these will not be discussed in detail in this report.

METHODS

For the determination of 50% lethal temperature (LT_{50}) and median burial temperature (BT_{50}) values experimental animals were collected from suitable field populations and transferred to acclimation tanks in the laboratory. After two days groups of animals from each acclimation temperature were transferred to each of a series of experimental tanks providing temperatures expected to extend well above and below the LT_{50} value. The numbers of animals dead and, where appropriate, the numbers buried, were then assessed at intervals of 3h, 6h, 12h, 24h, 48h, 72h and 96h following initial transfer, and LT_{50} and BT_{50} values for each time interval determined graphically. Groups of animals were kept in the acclimation tanks as controls, and where there was excessive mortality in these controls, as happened in isolated cases only, the results were rejected. Suitable modifications of the basic techniques allowed the determination of lethal temperatures for both macrofaunal and meiofaunal species. As far as possible the techniques used and the criteria for assessing mortality in the different species were standardised between Oban and Marseille, so that the results obtained for northern European and Mediterranean animals could be reliably

compared.

At Oban, determinations of upper lethal temperature limits (LT_{50}) were made for 14 species from the macrofauna and 4 species from the meiofauna of intertidal and subtidal soft sediments from the Scottish west coast. At Marseille, similar determinations were made for 10 species from the macrofauna and 5 species from the meiofauna from soft sediments from the Mediterranean coast of France. For some species a single determination only of lethal temperature was made, but for most, the effect of acclimation temperature was examined by repeating the determinations at intervals during the year, at times when the animals were naturally acclimated to different environmental temperatures. Observations on the burrowing response involving the determination of BT_{50} values were made for the same macrofauna species where appropriate (i.e., for those which normally live buried in the sediment).

A tabulation of LT_{50} and BT_{50} values resulting from this work was given in the full reports on the contract. This report will discuss only the main conclusions and generalisations which arise from the results.

MACROFAUNA RESULTS

For all the species examined the change with time in LT_{50} followed a similar pattern. A fairly steep decline in LT_{50} over the first 24-48 h was followed by a more slowly declining or stable LT_{50} up to 96 h. The change in BT_{50} with time showed a generally inverse pattern to that of LT_{50} although in most cases a more or less constant BT_{50} value was reached within 24 h of the start of the experimental period. In some cases, BT_{50} values increased up to 12-24 h but then decreased, indicating the return of individuals to the surface in a stressed condition prior to death.

Two major variations from the general pattern occurred. In one, represented by the case of the bivalve Cardium edule examined at Oban, LT_{50} and BT_{50} values continued to decline steeply beyond 24 h, indicating a secondary mortality affect in the experimental conditions possibly resulting from the build up of metabolites in the waters. In the other variation, represented by the gastropods examined, the BT_{50} values were very irregular as a result of the occurrence of circadian rhythms of activity in these animals.

The results showed a range of 24 h LT_{50} values for $10^{\circ}C$ acclimated animals for the macrofaunal species examined from the Scottish coast between 21° and $33^{\circ}C$, and for the more restricted variety of species examined from the Mediterranean coast, a range of 24 h LT_{50} values for 15° acclimated animals between 29° and $33^{\circ}C$.

The influence of certain environmental and other factors on the LT_{50} and BT_{50} values found for the macrofauna was examined and the results suggest the following general conclusions.

a) Acclimation temperature and other seasonal affects.

Seasonal acclimation to the temperature of the environment, reinforced by a short period of experimental acclimation in the laboratory, resulted in an increase in LT_{50} and BT_{50} values, with increasing temperature. The intensity of this effect was similar in most of the species examined, with LT_{50} values increasing approximately $0.1-0.14^{\circ}C$ per degree centigrade difference in acclimation temperature. In general the increase in BT_{50} values was greater than for LT_{50} values. Certain species, however, showed significantly smaller increases with increasing acclimation temperature indicating a lower sensitivity to temperature shock in these species.

One species, Cardium edule, showed a normal acclimation effect for LT_{50} values up to 24 h, and thereafter a reversed acclimation effect. The secondary factor affecting mortality in this species, which was also noted in relation to the time course of change in LT_{50} values, is itself affected by acclimation temperatures although in this case the mortality associated with the secondary factor is greater at higher acclimation temperatures.

b) Age of individuals

The effect of differences in age of individuals on LT_{50} and BT_{50} values were tested for Donax trunculus from the Mediterranean by comparing values for animals from two modal groups within the population, one of 10.3 ± 0.3 mm, the other of 27.9 ± 1.2 mm. There was no significant difference in this case in LT_{50} between the two groups but there was a clear difference in the burrowing response, the young reacting more rapidly than the adults.

c) Depth distribution

The observations made at Oban and Marseille both concerned groups of species which show clear differences in their normal range of

distribution in relation to depth of water, and the LT_{50} values and BT_{50} values found show consistent correlations with depth distribution. In each case, where two closely related species distributed in the same area but at different depths were compared, the species having the shallowest distribution had the higher temperature tolerance as indicated by LT_{50} and BT_{50} values. This relationship was found for the bivalves Tellina tenuis and T. fabula from Scottish beaches, Donax trunculus and D. semistriatus from the Mediterranean, and the gastropods Nassarius pygmaeus and Cyclonassa neritea from the Mediterranean.

For less closely related species distributed at different depths there was also a rough but significant relationship between temperature tolerance and the distribution of the species with relation to tidal exposure, or depth of distribution. In general those species whose range extends further shorewards showed the highest values of LT_{50} and BT_{50} .

d) Influence of metabolism

Where a number of species of similar distribution pattern have been examined there is a tendency for the species with the highest metabolic rate to have the lowest thermal tolerance. Certain species therefore show a higher thermal tolerance than would be expected from their normal depth of distribution. This was the case for Corbula gibba and Venus gallina in the Mediterranean and Venus striatula from Oban. The same species also showed a lower sensitivity to temperature shock as indicated by the reduced effect of acclimation temperature on LT_{50} values noted earlier.

e) The influence of geographical distribution

The influence of geographical distribution on the temperature tolerance was investigated by comparing LT_{50} and BT_{50} values determined in Marseille and Oban for species which have a wide range of distribution in European waters and occur on both Mediterranean and Scottish coasts, and for groups represented by two or more closely related species of more limited geographical range in the two areas. The main comparisons concerned bivalves of the genera Tellina, Donax, Venus, Cardium, Spisula and Corbula, and gastropods of the family Nassariidae. Where populations of the same species from Mediterranean and Scottish waters were compared the southern populations showed a higher temperature

tolerance with a difference in LT_{50} values for the same acclimation temperature no greater than 2°C . Where pairs of closely related species occupying the same biotope were compared the species having a more southerly distribution showed a higher temperature tolerance. In this case the difference in temperature tolerance could exceed 2°C . Such comparisons are valid only where the biotopes are similar since differences in depth distribution may override differences of geographical distribution.

MEIOFAUNA RESULTS

Although less extensive than the results for the macrofauna, the results of the determinations of LT_{50} for meiofaunal harpacticoid copepods from Mediterranean and Scottish west coast sites lead to similar conclusions. With one exception, the relationship between LT_{50} values and time for the species examined at Oban showed a similar relationship to that found for the macrofauna. The exception was male Harpacticus flexus, and in this case, and in all the Mediterranean species examined, there was a continued decline in LT_{50} values beyond 24 h, indicating a secondary mortality similar in effect, but not in cause, to that found for Cardium edule among the macrofauna. The results for all determinations of the meiofauna show a range of 24 h LT_{50} values for 10° acclimated animals lying between 25° and 33°C .

a) Acclimation temperature and other seasonal effects

Only the Oban observations on LT_{50} values for the harpacticoid copepods provide sufficient data for the effects of seasonal acclimation temperature to be examined, and none of the species studied showed clear evidence of acclimation to the environmental temperature, although there were differences in some species between the LT_{50} values for animals acclimated to the same temperature at different dates. The results for Asellopsis intermedia (adult females) show a clear seasonal effect which cannot be explained simply as acclimation to the ambient temperature, and there are thus clearly seasonal variations in temperature tolerance, which can perhaps be related to the different characteristics of the life cycle in these short lived animals.

b) Depth distribution

Although a limited number of species were examined, the results found for the meiofauna confirm the conclusions reached from the

macrofauna results that the greatest temperature tolerance was shown by those species with the shallowest, or intertidal, distribution. Of the species examined at Oban, the most resistant species, Asellopsis intermedia, is restricted in its distribution to the intertidal zone, while the closely related species Asellopsis hispida which occurs mainly in the subtidal sands, has a much lower temperature tolerance. Species from both Mediterranean and Scottish west coast waters show a similar general relationship between temperature tolerance and depth distribution to that found for the macrofauna.

c) Effect of geographical distribution

The main conclusions reached for the macrofauna apply also to the meiofauna results when LT_{50} values for populations and species from the two areas are considered. In the one case, Harpacticus flexus, where populations of the same species from both Mediterranean and Scottish waters were compared the southern population showed a greater temperature tolerance, with a difference in LT_{50} values for the same acclimation temperature no greater than $2^{\circ}C$. When closely related species were compared, for example for Asellopsis species, the differences in LT_{50} found were consistent with the effects of depth and geographical distribution found for the macrofauna.

d) Comparison with macrofaunal species

Where both macrofauna and meiofauna species from the same habitat have been examined the meiofauna species has been found to have the greater thermal tolerance. Examples are the cases of Asellopsis intermedia and Tellina tenuis from intertidal sands on the Scottish coast, where the LT_{50} values for the harpacticoid are $1-2^{\circ}C$ higher than the bivalve under comparable conditions, and Asellopsis hispida and Tellina fabula from subtidal sands where the difference in LT_{50} values is $3-4^{\circ}C$.

DISCUSSION

Determinations of the lethal temperature limits (LT_{50}) for species potentially subject to thermal pollution serve two purposes, 1) to set the minimum temperature requirement above which survival of a given species in a thermally polluted area would be impossible, and more importantly, 2) to give a comparative background providing indications of where more subtle sublethal effects might first begin to show, since those

species with a high thermal tolerance might be expected to show greater thermal tolerances also in their physiological functions. The present series of determinations represent the first extensive study of the thermal tolerances of invertebrates from shallow soft sediments in European waters. They indicate the range of temperature within which lethal effects on at least some species would be expected for both macrofauna and meiofauna species.

Significant effects of thermal discharge plumes impinging on the shore are most likely to be shown in those animals distributed in the shallowest areas. We might expect therefore greater effects on intertidal communities of northern European coasts and on the shallow water communities of Mediterranean coasts, than on subtidal communities of northern coasts or the deeper communities of Mediterranean coasts. The present results suggest that the greater vulnerability of exposure to thermal additions to the environment in the shallower communities may be compensated by the greater thermal tolerance found in species from these areas. The results show also, however, that the scope for tolerance of additional thermal pollution is less in the Mediterranean than in northern European waters.

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Contractor: University College of Swansea
Contract No: 101-74-7 ENV UK
Project Leader: Professor J. A. Beardmore
Title of project: Assay of genetic damage by metals in
estuarine populations of Mytilus

Estuarine waters frequently contain appreciable concentrations of heavy metal compounds. Because of its mode of feeding Mytilus would be expected to reflect differential metal concentrations in sea water in the tissues of animals from different populations. Thus genetic damage due to heavy metals might be monitored in such populations.

Two approaches to monitor genetic damage have been used

1. Chromosome studies. Studies of the chromosomes of Mytilus have been unrewarding. A variety of somatic tissues produced very low frequencies of utilisable mitotic figures even with colchicine treatment. The use of phytohaemagglutinin was found to be helpful but the maceration of tissue involved produces a lack of discreteness in individual nuclear karyotypes.

Fertilised eggs and early zygote stages while providing an abundance of division phases exposed other problems. The main difficulties arose from 1) the limited spawning period of Mytilus and 2) the effect of the egg yolk which hinders squashing and effective staining. Study of chromosomes was therefore abandoned in the later part of the period.

2. Electrophoretic assay. Gel electrophoresis provides a system of moderately great resolving power and nine loci have been added to the four on which earlier reports are based (Table 1).

m-Aat	m-Amino aspartate transferase
s-Aat	s- " " "
Ap	Aminopeptidase
Est-D	Esterase-D
Est-mu	Esterase-muscle
m-Idh	m-Isocitrate dehydrogenase
Lap-1	Leucine aminopeptidase-1
Lap-2	" " -2
NAD-Mdh	NAD-Malate dehydrogenase
Me	Malic enzyme
6-Pgdh	6-Phosphogluconate dehydrogenase
Pgm	Phosphoglucomutase
Phi	Phosphohexoseisomerase

Table 1. Loci screened electrophoretically for rare variants.

Pooled samples of several individuals used with some monomorphic systems in the hope that single rare heterozygotes would be detected and that the total number of individuals sampled in unit time would be increased did not give useful results. The rationale of the electrophoretic approach depends upon monitoring the frequency of rare variants, some which may be the results of new (or more probably) recent mutations. Rare variants are those genes whose individual frequency is less than 0.01. In general populations of M.edulis have been used but some populations of M.galloprovincialis have also been sampled.

Tables 2 & 3 show the data on the incidence of rare variants for earlier work using 4 loci and later work using thirteen loci respectively.

No. of populations sampled	Metal concentration	No. of genes assayed	No. of rare variants	% Frequency of rare variants
6	High	20,912	60	2.869†
7	Medium	6,488	11	1.695†
7	Low	4,160	6	1.442†
Total		31,560	77	1.442†

† Daly's χ_1^2 for trend is significant at $P = 0.032$.

Table 2. Incidence of rare variants in populations of Mytilus exposed to different levels of heavy metals in the sea. The data are based on equal number of genes at the Ap, Lap, Pgm and Phi loci.

Population	Metal concentration	No. of genes assayed	No. of rare variants	% Frequency of rare variants
Mumbles (M)	High	19148	326	17.02
Milford Haven (MH)	?	5320	77	14.47
Solva (S)	Low	7616	81	10.63
M:S	$\chi^2_1 = 14.86$	P = <0.001	(M+MH):S	$\chi^2_1 = 12.92$
M:MH	$\chi^2_1 = 1.67$	P = >0.30		P = <0.001
MH:S	$\chi^2_1 = 3.82$	P = 0.05		

Table 3. Incidence of rare variants in populations of Mytilus and comparison of frequencies. The data are based on twelve loci (Est-muscle could not be typed in one sample).

The data on metal concentrations used for the metal index in Table 2 are drawn from published data. There is a significant trend of association between crude metal concentrations and frequency of rare variants in Table 2. In Table 3 three populations are represented. Mumbles and Milford Haven resemble each other in total frequency of rare variants and are both significantly higher than Solva. Table 4 shows that Mumbles animals contain much higher amounts of Cd and Zn than Solva animals and this is in line with water assays.

Metal	Digest	Locality		Dry Wt.
		Mumbles Dry Wt.	Solva Digest	
Cd	0.38 ± 0.02	1.78 ± 0.14	0.24 ± 0.016	0.74 ± 0.02
Zn	12.74 ± 2.17	61.09 ± 12.70	3.47 ± 0.25	10.78 ± 0.98
Cd	Mumbles:Solva dry wt.	t = 5.45	P = <0.001	
"	" digest	t = 7.31	"	
Zn	" dry wt.	t = 4.24	"	
"	" digest	t = 3.97	"	

Table 4. Heavy metal concentrations (p.p.m) in digests and dry weight of samples of Mytilus from Mumbles and Solva.

Precise figures are not available for Milford Haven though the levels are thought to be low.

Mutagenicity assay

Dr. J. M. Parry has shown that alcoholic extracts of Mytilus tissues (particularly mantle) from areas known to be polluted are mutagenic in several microbial test systems whereas mussels from clean water areas do not contain such mutagenic factors.

Suggestive evidence that aqueous extracts which include heavy metals are also mutagenic in microbial test systems has also been obtained.

Chemical assay

Dr. J.M. Ballantyne (Department of Chemistry, U.C.S.) has carried out partial analyses of mussels from a number of sites. The most striking difference between Mumbles mussels and those from clean water areas is that the former contain relatively large amounts of short chain hydrocarbons.

We conclude that while heavy metals show some association with the level of rare genes in Mytilus populations a causal relationship is not yet established. Two further points need to be made:-

1. The difference in frequency of rare variants could conceivably be a result of natural selection although the frequencies of common genes do not show parallel differences.
2. Waters containing high levels of metals are likely also to be contaminated with other materials particularly oil and products thereof.

Future work will be directed towards resolution of both of these points.

Publications: Mahmud Ahmad & J. A. Beardmore. Genetic evidence that the Padstow mussel is Mytilus galloprovincialis Marine Biology 35 139-147 1976.

Contractor: Ministry of Agriculture, Fisheries and Food
Contract No: 109-74-7 ENV UK
Project Leader: Dr I C White
Title of Project: ASSESSMENT OF THE ACUTE AND SUB-LETHAL EFFECTS OF VARIOUS POLLUTANTS ON SELECTED MARINE ORGANISMS AND THE FATE OF PETROLEUM HYDROCARBONS IN AN ESTUARY SUBJECTED TO A REFINERY EFFLUENT.

- a) The development of improved methods of testing the acute toxicity to marine organisms of persistent and non-persistent mineral oils, oil-dispersants and oil/dispersant mixtures.

A suitable system using cylindrical Perspex tanks fitted with central stirrers has been devised to maintain oils, oil dispersants and mixtures of the two as an homogenous dispersion of small droplets or particles throughout the water column, at concentrations up to 10^4 parts per 10^6 . This apparatus has been used to test a number of non-persistent oils but problems were encountered, especially the reduction of dissolved oxygen levels in the sealed tanks. The apparatus has, however, been used extensively to determine the toxicity to Crangon crangon of a wide range of oil dispersant products mixed with oil and these results have been compared with those obtained for oil alone and dispersants alone under static conditions. A separate test has also been developed to simulate the conditions experienced by inter-tidal animals when oil dispersants are used on beaches. This test relies on the exposure of Fatella vulgate to neat dispersant in air prior to reimmersion in water and the results for a wide range of products have been compared with those obtained under static test conditions using Crangon.

- b) Assessment of long-term sub-lethal effects of marine pollutants on selected marine organisms.

The ability of animals to reproduce successfully was identified as a suitable significant parameter for experimental study of the effect of pollutants. After careful consideration the American Slipper limpet, Crepidula fornicata (L) was selected as a suitable test species and the conditions for its successful rearing and breeding under laboratory conditions have been determined. Initial experiments have related changes in the condition of adults, established by different feeding regimes, to changes in weight, biochemistry, fertility,

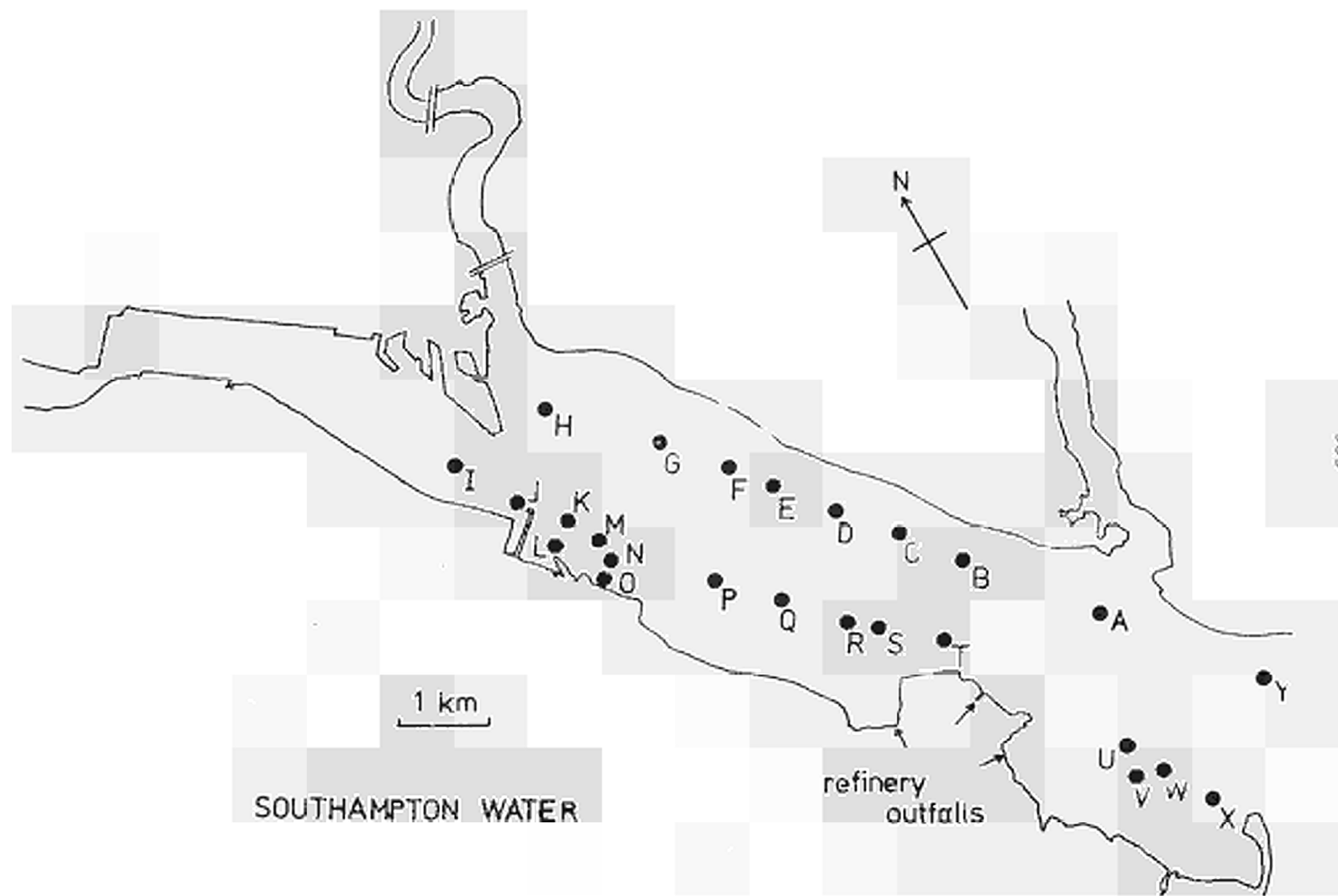
fecundity of adults and viability of larvae produced. The effects of a pollutant on these basic parameters is now planned. Populations of Crepidula in areas of different water quality have been identified for study. These will be compared to the laboratory maintained populations.

- c) The fate of petroleum hydrocarbons in an estuary subjected to a refinery effluent, with reference to their distribution in commercial species of marine organisms.

This pilot project has been carried out to assess the feasibility of establishing the pathways by which petroleum hydrocarbons are incorporated into marine sediments and organisms within an estuary receiving a quantifiable refinery effluent. Close co-operation has been maintained with other workers in the area, especially Southampton University, and their analyses of the refinery effluent, sampled regularly during 1975 and 1976, has illustrated the range of hydrocarbons present and their variability in quantity and composition. Further effort has been devoted to the determination of the volatile hydrocarbons in the effluent which may account for apparent differences in the amount of "oil" discharged as determined by different methods. Effort has also been devoted to obtaining samples of sediments and biota for hydrocarbon analysis, the development of suitable analytical techniques and the solution of a number of associated problems which delayed the production of the results of this initial survey. However these difficulties have been overcome and both sediment and benthos samples have been fully analysed. These indicate that additional significant sources of petrogenic hydrocarbons are present in the estuary which obscure any clear relation between hydrocarbon contents of sediment, benthos and the refinery effluent. Benthos hydrocarbon contents only partially reflect those of their sediments, both in quantity and composition. Further sampling and analysis is planned to study potential additional inputs and analytical techniques suitable for detailed characterisation of the refinery effluent will be examined.

SOUTHAMPTON WATER, JUNE 1975
HYDROCARBON ANALYSES ($\mu\text{g g}^{-1}$ wet weight)

		HYDROCARBON FRACTIONS					
TYPE	STATION	F1	F2	F3	F4	F5	$\Sigma_1^5 F$
SEDIMENT	A	33.3	42.4	8.7	2.4	6.3	93
	B	4.1	15.2	2.7	2.6	3.7	28
	C	27.0	75.8	6.2	4.3	4.3	118
	D	7.1	114.3	10.2	3.7	5.0	140
	E	1.5	62.0	2.5	6.5	4.5	77
	F	6.6	91.5	5.9	8.8	6.2	119
	G	22.1	80.8	5.9	4.5	4.1	117
	H	57.0	3.2	5.0	3.1	1.9	70
	I	12.8	156.6	7.6	5.7	4.0	187
	J	200.5	4.5	3.6	3.3	4.1	216
	K	6.1	284.0	19.6	18.4	5.2	333
	M	73.5	49.0	1.5	2.5	6.4	133
	P	5.0	505.0	36.1	29.8	6.1	582
	Q	3.8	7.4	4.9	18.6	243.5	278
	R	5.7	215.9	7.8	11.1	4.0	245
	S	47.3	150.0	7.3	8.4	4.9	218
	T	5.3	168.5	3.7	8.8	0	186
	U	8.0	5.0	11.0	6.3	7.8	38
	V	54.0	116.8	8.0	9.1	12.1	200
W	40.3	77.5	7.3	5.8	1.7	133	
X	61.4	59.3	6.0	8.7	5.5	141	
Y	7.7	14.0	3.8	1.5	3.4	30	
MERCENARIA	A	5.0	4.2	3.8	1.4	2.6	17
	B	1.2	1.5	0.9	0.8	1.2	6
	C	3.4	13.9	3.9	1.5	3.3	26
	E	4.5	6.9	4.4	1.6	1.7	19
	G	6.3	2.8	3.5	6.2	4.4	23
	H	4.1	12.6	17.0	14.4	7.7	56
	I	5.5	18.4	2.3	9.5	4.1	40
	L	5.7	5.4	5.6	2.4	3.4	23
CARDIUM	J	7.7	6.4	0.9	7.4	4.4	27
	K	8.8	17.6	1.3	11.0	7.0	46
	L	6.9	3.9	4.5	2.9	3.4	22
	P	8.4	5.0	9.2	7.0	0.8	30
CREPIDULA	L	13.4	19.9	14.6	15.4	16.7	80
	X	32.0	31.0	9.8	17.8	5.0	96
	X	28.3	22.2	9.1	7.6	24.6	92
	Y	20.0	19.4	16.8	4.4	3.0	64
BUCCINUM	Y	22.1	28.4	14.0	4.6	3.6	73
OSTREA	X	7.7	19.1	9.6	9.7	13.4	60
EUPAGURUS	P	21.0	135.4	8.3	2.9	41.5	209
	U	5.9	43.0	8.0	14.5	13.1	85



Contractor : University College, Dublin
Contract No.: 095 - 74 - 11 Env. Eir.
Project Leader : Dr. D. A. Murray
Title of Project : Paleolimnological Investigations on
the Sediments of L. Ennell and L. Owel.

The object of this research has been to determine the rate of eutrophication in two Irish lakes by an examination of vertical cores of sediment. Materials which enter a lake from the water-shed and the atmosphere come under the control of lacustrine transport mechanisms, may be utilized in intra-ecosystem cycles, and eventually are incorporated into the sediments on the lake floor. This accumulated sediment together with material derived from sources within the lake, e.g. remains of organisms etc. constitutes a chemical and morphological fossil record.

The two lakes investigated (in the current study) are of synchronous origin and are separated by a distance of approximately 11 Km. Lough Ennell, a eutrophic lake, has, since 1972, supported persistent growths of algae and has a mean secchi disc transparency of 1.0 m. In contrast, Lough Owel is a moderately eutrophic clear water lake with a transparency of up to 8.0 m. It has been suggested that the present condition of L. Ennell is due to the discharge of sewerage from the nearby town of Mullingar (population 10,000).

Summary of Results

- i) Pollen analysis and chronology - Pollen was analysed in the L. Ennell sediment only, resulting in the establishment of a time scale for the core. Three pollen assemblage zones have been distinguished and correlated with some modern pollen diagrams from central Ireland. Horizons at 1700/1750 AD (25 cms), 1600 AD (40 cm), 1400 AD (60 cm) and 1000/1100 (140 cm) are recognised.

Analysis of pollen in L. Owel was not attempted since similar features in the chemistry profiles of cores in both lakes are detected which may be related to cultural activities.

An analysis of ^{137}Cs on sediments from both lakes gave estimation of the sedimentation rate which in L. Ennell is 7.0 mm, 1.75 times the rate for L. Owel - 4.0 mm. The calculated rates for the last 900 years show that the sedimentation rate has been consistently greater in L. Ennell - in earlier times, however, only a factor of 1.4 compared to the recent 1.75. The persistent difference in sedimentation rate may in part be related to the extent of the catchment area which is 3.7 times greater around L. Ennell than around L. Owel. However, the significantly different value in recent sedimentation rate is undoubtedly due to an increase in autochthonous deposition as a result of increased productivity in L. Ennell.

ii) Productivity Studies

Information on past primary productivity is obtained from sedimentary pigment analysis. In Lough Ennell, concentrations are relatively uniform up to the 30 cm level. From this level, however, pigment concentrations increase significantly and although fluctuations in pigment concentrations occur, the lowest value detected here is more than twice that in the older sediments. The pigment profiles in L. Owel differ from L. Ennell and indicate that up to approximately 1300 AD, L. Owel may have been more productive than L. Ennell. In the last 200 - 300 years, however, while pigment concentrations increase rapidly in Ennell which has a mean value of 225 arbitrary units, the concentrations in L. Owel are relatively low at a mean value of approximately 100 units. The ratio of chlorophyll derivatives to total carotenoids (D 665/480 mu), used as an index of trophic status, confirms the trends observed in pigment concentrations. For approximately the last 900 or 1000 years, L. Ennell has undergone a progressive increase in the rate of eutrophication and this rate is seen to have changed most significantly in the last 10 - 20 years. In marked contrast the trophic status of L. Owel appears to have remained relatively stable since the 15th century.

iii) Microfossil analysis

- a) Chironomid remains - the ecological specificity of the larvae of these insects has given rise to many attempts to interpret the typological succession of lakes by studying their chitinous remains in

sediment cores. Analysis of microfossils in cores from both lakes show that L. Ennell has been moderately productive throughout the period of time represented in the core. The average number of head capsules deposited/cm² per year has increased from 1.87 prior to 1700/1750 to 5.18 at the present time. The data suggests that the most significant changes took place in the last 15 - 30 years. The cores from L. Owel show a relatively uniform distribution of types throughout the last 1000 years. It is noteworthy, however, that head capsules of Chironomus larvae are approximately 2% more numerous in L. Owel in the old sediments, suggesting that oxygen depletion has occurred in the past in L. Owel concurring with views expressed above.

- b) Cladoceran remains - Twenty-three species of Cladocera were identified from cores in L. Owel and twenty-five in L. Ennell, where a gradual increase in numbers is noted and in the recent sediments, a density of 3500/gm organic weight is observed. The chydorid stratigraphy in L. Ennell, especially during the last 400 years, shows significant changes indicating a radical alteration of trophic status. Chydorus sphaericus, normally associated in high numbers with enriched or polluted waters increases from 5% representation at the 50 cm level (approx. 1500/1600 AD) to current 15% representation, while concurrently species primarily associated with unproductive waters exhibit a decreasing trend, e.g. Alonella excisca decreases from 15% to 2%. The chydorid stratigraphy in L. Owel shows some similarities, e.g. here also an increase in numbers of Chydorus sphaericus is seen, but in contrast maximum abundance of cladoceran remains - approximately 3000 per gram organic weight is seen at 60 cm (approx. 1100 AD) and during the last 500/600 years numbers have remained relatively uniform (average 1000/gm organic weight).

Chemistry of the cores - Profiles of Ca, Na, Mg, K and mineral content indicate that throughout the period of time represented in the cores erosion from the catchment has varied in intensity but has been relatively high. There is a good correlation between the peak amounts of these elements in the sediment and evidence of deforestation obtained from the pollen analysis. This trend is most noticeable with the K profile. The profiles for Mn and Fe show interesting features, especially in the upper 20 cm of L. Ennell sediment. Relative concentrations of these elements reflects aerobic or anaerobic conditions in the hypolimnion. During the last 180-200 years, L. Ennell underwent periodic anoxic conditions as indicated by a decrease in concentrations of Manganese. In contrast, in L. Owel both Fe and Mn show increased concentrations in the more recent sediments, while in the older sediments there are indications that de-oxygenation did occur. The trends observed in the P. profile are

indicative of increasing nutrient input into the systems. There appears to be a significant correlation between the amounts of available phosphate and the concentrations of sedimentary pigment recovered.

General Conclusions

Examination of sediment cores from L. Ennell for chemistry, sedimentary pigments and microfossils indicate that the lake has been evolving towards a eutrophic condition at least for the last 900 years. The changes which have occurred may be related to cultural activities in the catchment area. It is noteworthy that the rate of eutrophication has most significantly increased in the last 100 years. Increase in primary productivity is indicated by increase in sedimentary pigment concentrations and changes in the cladoceran and chironomid communities. This increase is correlated with an increase in phosphorus.

Sediment cores in L. Owel reflect some trends differing from L. Ennell. It is probable that in earlier times L. Owel was as productive, if not more productive than L. Ennell in its natural state and up to the 14th or 15th century both lakes were evolving simultaneously in terms of productivity. From that time on, the eutrophication process in L. Owel appears to have stabilized or decelerated. In L. Ennell, however, the rate of eutrophication has continued to increase and outstripped L. Owel. This has resulted in lakes of a vastly different character today and it is suggested that human cultural activities in the catchment area of L. Ennell have been directly responsible for this situation.

It is apparent that a considerable period of time was required before a eutrophication problem became apparent and based on the results obtained it is now suggested that coring studies may provide useful information of impending eutrophication.

Publications

- Murray, D.A. and D.J. Douglas, 1976. Some sedimentary pigment determinations in a 1.0 metre core from L. Ennell, a eutrophic lake in the Irish Midlands - Proceedings of the International Symposium on Paleolimnology and Paleoclimate in Paleolimnology of Lake Biwa and the Japanese Pleistocene. S. Horie Ed.
- Murray, D.A. and D.J. Douglas, 1977. Eutrophication Past, Present and Future? - Proceedings of Seminar on Eutrophication, Killarney. National Science Council of Ireland.

TOPIC 5 : REMOTE SENSING OF AIR POLLUTION

Contractor : Fraunhofer-Gesellschaft zur Förderung der
Angewandten Forschung e.V., Munich, Germany

Contract No: 013-74-1 ENVD

Project Leaders: Dr. R. Reiter and Dr. W. Carnuth

Title of Project: Absolute Calibrated Double Frequency
Lidar for Remote Aerosol Sensing

1. Technical Development of the Lidar System

An existing stationary, vertical pointing lidar system with a 100 MW, 694 nm wavelength ruby laser and 52 cm dia. receiving telescope has been extended by a KDP frequency doubler, a second interference filter etc. for additional 347 nm wavelength operation. Using two transmitter wavelengths it was intended to get information about both aerosol particle number concentration and size distribution. For the digital recording of the lidar return signals a Biomation 8100 transient recorder is used. The data are output on punch tape for further computer processing. Lidar signals from altitudes above 6 km are measured by a ten-channel photon counter with range gating. An electromechanical chopper rejects the incoherent ruby fluorescence which otherwise would interfere with the faint high-altitude signals. For analog measurements the range gating facility also avoids any DC overloading of the photomultiplier tube due to the background luminosity, thus allowing sufficient signal amplification by applying higher voltages to the tube alone, without using any additional amplifier, which results in improved signal accuracy.

2. Theoretical Procedures

For the experimental calibration of the system and the derivation of the aerosol data from the backscatter profiles extensive mathematical procedures have been developed. As only two transmitter wavelengths are available with the lidar system, the particle size distribution as measured by five-stage impactors at 750, 1800, and 3000 m a.s.l. is approximated by a bimodal model consisting of two log-normal dis-

tributions ("coarse" and "fine" particles) with constant half-widths and center diameters, but with the two integrated number concentrations as variable parameters. The derivation of these two number concentrations is the goal of the mathematical procedures and calibration measurements. At first total (molecular plus aerosol) backscatter coefficients are calculated for the three station levels. The molecular part of the total backscatter (Rayleigh scattering) is proportional to the number density of the air molecules as measured by stationary and mobile (cable car and balloon-borne) probes. The aerosol part is calculated from the impactor data using Mie's theory. Starting with the calculated backscatter coefficient for the 1800 m level, and using the measured backscatter profiles, the lidar equation is then solved for both wavelengths in a step-by-step method accounting for the integral extinction term. The procedure results in particle concentration profiles for the fine and coarse size range, and in system calibration constants for both wavelengths. The degree of agreement between the calculated and measured concentrations at the two other station levels, e.g. at 750 and 3000 m, is an indication for the reliability of the calculations.

3. Tropospheric Analog Measurements

During the two years of contract about 470 two-frequency analog lidar returns have been recorded and stored on punch tape. Due to a delay in the calculation work arising mainly from the necessity of developing double-precision arithmetics, only a small part of the data could be evaluated during contract time. One preliminary example for calculated profiles of particle concentrations in the fine and coarse size range is shown in Fig. 1 together with the backscatter profiles in the two wavelengths (B_R and B_{UV}). In the left half of the diagram, profiles of aerological parameters measured by cable car probe are shown for illustration of the meteorological situation, viz., temperature, T , wind speed, and polar electric conductivities, λ_+ and λ_- , giving an indirect measure of the particle number concentration.

On the right hand, the relative backscatter intensities in the two wavelengths (red, B_R , and ultraviolet, B_{UV}) are plotted. The concentration profiles for the coarse and fine particles have been calculated starting from the impactor data at 1800 m altitude (W_1 and W_2). Z_1 and Z_2 denote the results of the impactor measurements at 3000 m, which fit fairly well to the extrapolated calculated number concentrations at that level. The absence of fine aerosol data above 2100 m as well as the gap in the coarse particle profile just below are caused by the occurrence of negative particle concentrations during the course of the evaluation routine, i.e. the calculated total backscatter function was smaller than the Rayleigh part. Experiences of this kind reveal the need of improving the measurement accuracy especially in the 347 nm wavelength. In the meantime, after finishing of the contract, the data acquisition electronics has been extended by an on-line computer, allowing rapid data transfer from the Biomation recorder and thus averaging any number of return signals within a short time for signal noise reduction and increased accuracy.

4. High Altitude Measurements By Means of Photon Counting

Reliable stratospheric lidar measurements have been made possible at the end of contract. An example is shown in Fig. 2. In a semi-log scale, range-corrected backscatter profiles obtained by photon counting (right) and analog recording (left) are plotted versus altitude. The straight line presents the molecular density profile measured by radiosonde. The measurements were made with the fluorescence rejection device in operation. For comparison, from 30 to 32 km altitude a measurement was carried out without rejecting the ruby fluorescence. The result is included in the diagram and reveals the fluorescence suppression to be absolutely necessary.

5. Publications

- 1) Reiter, R., and Carnuth, W.: Comparing Lidar Reflectivity Profiles against Measured Profiles of Vertical Aerosol Distribution Between 1 and 3 km a.s.l. Arch. Met. Geoph. Biokl. A 24, (1975).

- 2) Reiter, R., et al.: Analysis of Aerosol Transport. Final Technical Report 1975, Grant Agreement No. DA-ERO-591-73-G0057, Annual Report 1976, Grant Agreement No. DA-ERO-75-G-077.
- 3) Reiter, R., and Carnuth, W.: Remote Aerosol Sensing with an Absolute Calibrated Double Frequency Lidar. 22nd Technical Meeting of AGARD on Optical Propagation in the Atmosphere, Lyngby, Denmark, 1975.
- 4) Reiter, R., et al.: Technical Layout, Theoretical Basis and Calibration Procedures of Multifrequency Lidar Systems Applied for Remote Aerosol Sensing. Symposium on Radiation in the Atmosphere, Garmisch-Partenkirchen, Germany, 1976.
- 5) Reiter, R., et al.: Results of Remote Aerosol Sensing in the Troposphere and Stratosphere. Symposium on Radiation in the Atmosphere, Garmisch-Partenkirchen, Germany, 1976.

Fig. 1: Profiles of fine and coarse aerosol concentration calculated from lidar backscatter profiles

Fig. 2: Stratospheric lidar backscatter profiles measured by photon counting (right) and analog recording (left)

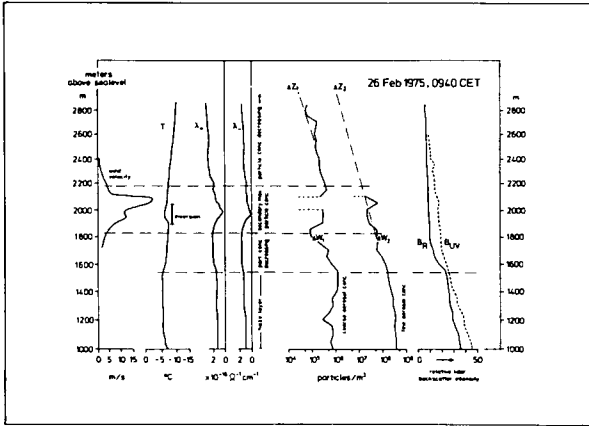
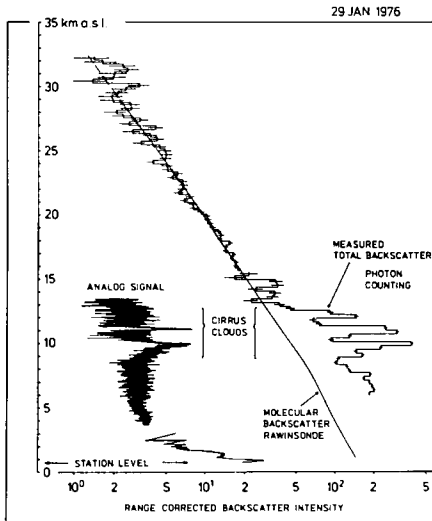


FIG 1

FIG 2

LIDAR BACKSCATTER PROFILE AND COMPUTED MOLECULAR RETURN



Contractor : Sektion Physik der Universität München

Contract n^o 064-74-1 ENV.D.

Project leader : J. Brandmüller and W. Kiefer*

Title of project : Resonance Raman Scattering as a Method for
 Remote Sensing in Air

It is well known that Raman scattering where the exciting frequency is in resonance with electronic transitions of the molecule yields strongly enhanced intensities of certain Raman bands. However, the application of the resonance Raman effect to remote sensing of pollutants in air is limited to the scattering process where resonance takes place with the absorption continuum. Although scattering in resonance with discrete vibrational-rotational energy levels (often called resonance fluorescence) would result in much higher scattering cross sections than those obtained from scattering in resonance with the continuum, the former method has a severe drawback: Discrete level resonance scattering is characterized by a fairly long scattering time due to long lifetimes of the intermediate energy level which in most cases is of the order of micro-seconds. This makes ranging impossible and also forces one to use long gating times which on the other side do not allow to make remote scattering measurements during day time. If quenching of long-lived fluorescence by foreign gas molecules (e.g. N₂, O₂) occurs, the total amount of scattered resonance light is reduced appreciably. Because of these facts we have concentrated our study to the continuum resonance scattering where the total scattering time is of the order of 10⁻¹³ sec.

A Raman system consisting of a one-meter double monochromator, a 4-Watt (all lines) argon ion laser and a 100 MHz - photon counting system was set up and the sample area was modified in order to observe the scattered Raman light in a 90° as well as a 180° backscattering configuration. These arrangements allowed experimental studies of the influence of the absorption of the scattering gas on the observed Raman

signal when resonance excitation with the absorption continuum above the dissociation limit of the electronic excited state was employed. The amount of absorption of the laser line by the resonance scattering gas (e.g. Br_2 or I_2) was derived from measurements at various gas pressures where the path length between focused laser beam and cell window was varied (90° scattering configuration). In the 180° back-scattering arrangement similar measurements were made for bromine and iodine vapor. In both configurations it was found that the attenuation of the actually observed Raman signal which is due to the absorption of the laser beam as well as due to the re-absorption of the scattered resonance Raman signal can be described exactly by Lambert's law.

For some gases (e.g. I_2 and Br_2) showing continuum-resonance scattering in the visible region, we measured the scattering cross sections σ relative to the N_2 vibrational line (non-resonance) with several excitation frequencies. The strongest resonance enhancement was found for the most absorbing gas under investigation (I_2 , absorption coefficient $\epsilon = 420 \text{ l}\cdot\text{mole}^{-1} \text{ cm}^{-1}$ at the exciting frequency), for which σ turned out to be 198 compared to 1 for N_2 . For bromine vapor with much lower absorption ($\epsilon = 35 \text{ l}\cdot\text{mole}^{-1} \cdot \text{cm}^{-1}$ at the exciting frequency) the scattering cross section at maximum resonance was determined to be 17,5.

Laboratory measurements where a remote configuration was simulated were carried out to determine the detection limit for the fundamental vibration of a strongly resonating gas (I_2) which was found to be of the order of 30 ppb.

Summarizing the results of the resonance experiments in the laboratory and extrapolating these results to a Raman Lidar system, we obtain, using the following equipment:

laser: 0,2 J, 20 nsec
 collecting mirror: 1 m diameter
 monochromator: 40 % transmission
 photomultiplier: 40 % quantum efficiency
 range distance: 200 m
 gate interval: corresponding to 10 m range resolution (cloud diameter)

1. for non resonance excitation (e.g. N_2)

0,06 photoelectrons per laser shot per ppm

2. for resonance excitation (enhancement 200, extinction coeff.

$0,045 \text{ torr}^{-1} \text{ cm}^{-1}$):

- a) for 1 ppm: 12 photoelectrons per laser shot
- b) for 10 ppm: 85 photoelectrons per laser shot
- c) for 100 ppm: 40 photoelectrons per laser shot

In cases a) to c) the absorption of the laser light and the backscattered Raman light only within the cloud was taken into consideration. However, if the area between cloud and Lidar-system location is also absorbing the signals reduce appreciably: cloud 100 ppm, environment 10 ppm results in 0,04 photoelectrons per pulse.

Thus it follows from this study that the resonance Raman effect may increase the sensitivity of a Raman Lidar experiment considerably under the following conditions:

1. a laser with a pulse energy of the order of 0,01 to 0,1 J (or more) is available at a wavelength within the absorption continuum of the pollutant molecule.
2. the extinction coefficient of this molecule at the resonance wavelength is of the order of $\epsilon=10^3 \text{ l}\cdot\text{mole}^{-1}\cdot\text{cm}^{-1}$. The optimum remote resonance Raman signal is then obtained when $\epsilon\cdot c=1/d$ where c = concentration of the pollutant gas and d = cloud diameter. Thus, for $d = 10 \text{ m}$ and $\epsilon=10^3 \text{ l}\cdot\text{mole}^{-1}\cdot\text{cm}^{-1}$ c_{optimum} corresponds to 22,4 ppm.
3. the pollutant gas is concentrated in a cloud or plume at the emission source and not yet dispersed in a greater distance from the source.

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Publications prepared within the contract:

1. "Resonance Raman Lidar: A Laboratory Study", J.G. Hochenbleicher and W. Kiefer in Proc.Fifth Int. Conf. Raman Spectrosc., E.D. Schmid, J. Brandmüller, W. Kiefer, B. Schrader, and H.W. Schrötter, Eds., H.F. Schulz Verlag, Freiburg 1976, p. 428.
2. "A Laboratory Study for a Resonance Raman Lidar System", J.G. Hochenbleicher, W. Kiefer, and J. Brandmüller, Appl.Spectrosc. 30, 528 (1976).

Contractor : Messerschmitt-Bolkow-Blöhm, München
Contract n° : 090-74-1 ENV D
Project Leader : Mr. Rother
Title of project : Development of a two wavelength flash
lamp pumped dye laser

For a Lidar built at the Joint Research Centre, Ispra, it was asked to build a laser source emitting simultaneously two wavelength beams in the visible range which could be tuned separately over 30 nm each. The power output, pulses durations, wavelength ranges were fixed by the JRC according to the characteristics of the differential absorption measurements to be done with the Lidar.

The solution developed by MBB consisted of two dye cells made of two mirror halves to be exchanged underneath a flash lamp in another mirror half to make a complete optical resonator. As also two dye-circulation systems exist, it is possible to have different wavelength ranges. There is a light frequency doubler for producing a wavelength beam around 300 nm. For any dye cell, the beam is splitted by a Glan Thompson prism into two orthogonally polarized beams which are tuned independently. The tuners are made of six equilateral prisms of glass to be turned in such a way that comes out only the light wavelength for which the beam impinges onto the prism surface at the Brewster angle. The polarization planes are made parallel again by a special plate put in one beam.

All the parts of this laser source have been built and checked. Some are working normally but some electronic parts are not stable and reliable enough. Improvements are necessary before the laser source can be used in the Lidar.

Contractant : Commissariat à l'Energie Atomique
Centre d'Etudes Nucléaires de Grenoble

N° du contrat : 050 - 74 - 9

Chef du projet : J. COMERA

Titre du projet : Détection et Mesure de Polluants Atmosphériques
au moyen de l'absorption d'un faisceau laser
infrarouge à double fréquence

INTRODUCTION

Au cours de la dernière décennie, la possibilité d'effectuer une surveillance continue en temps réel des polluants atmosphériques par l'étude de l'absorption par ces gaz d'un faisceau laser s'est précisée et imposée.

L'existence d'une fenêtre atmosphérique autour de 10μ , dans laquelle de nombreux gaz ont des bandes d'absorption, et qui coïncide avec la bande d'émission des lasers à Co_2 dont la technologie est bien connue, ont imposé les conditions de ce travail.

Le but de cette étude était de mettre en pratique sur le terrain l'appareillage et la méthode déjà conçue au laboratoire.

Le principe de la méthode est le suivant :
au voisinage de $10\mu\text{m}$, l'absorption du rayonnement infrarouge par la vapeur d'eau et le CO_2 , ainsi que la diffusion par les aérosols et les poussières sont des fonctions lentement variables de la longueur d'onde. L'absorption par un polluant présente, par contre, des pics étroits. Donc, en comparant les atténuations du rayonnement aux longueurs d'onde λ_2 hors du pic d'absorption du polluant mesuré, et λ_1 , comprises dans celui-ci, on éliminera l'effet de l'atmosphère non polluée. On déduira donc la concentration moyenne sur le trajet des faisceaux du polluant étudié, de l'absorption différentielle aux deux longueurs d'onde, si on connaît ses coefficients d'absorption à λ_1 et λ_2 .

APPAREILLAGE UTILISE

La source de rayonnement est un laser à Co_2 d'une puissance de quelques watts qui, grâce à un dispositif breveté, émet séquentiellement (fréquence de l'ordre de 50Hz) deux faisceaux de longueur d'onde séparément réglables et confondus dans l'espace. Ces faisceaux, agrandis par un afocal (13 cm d'ouverture) afin de réduire la divergence, se réfléchissent sur un miroir plan (ϕ 25 cm) situé à quelques centaines de mètres de la source et sont recueillis par un miroir concave (ϕ 25 cm) qui les focalisent sur un détecteur pyro-électrique.

Les puissances émises sont aussi mesurées afin de tenir compte de leurs fluctuations. Le trajet maximum réalisé est de 1000 mètres.

Une électronique simple calcule analogiquement en temps réel la concentration moyenne C_a du polluant sur la base de mesure. L'alignement du trajet est effectué avec un laser à He Ne.

Un exemplaire de cet appareil a été construit pour les mesures sur le terrain, et installé à bord d'une caravane.

DETERMINATION DES COEFFICIENTS D'ABSORPTION

Les spectres d'absorption de gaz obtenus avec les spectroscopes classiques ne sont pas suffisamment résolus pour mesurer les coefficients d'absorption aux longueurs d'onde des raies laser.

Nous avons donc mesuré ces valeurs en plaçant sur le trajet des faisceaux une cellule contenant le gaz étudié à faible pression (10 mb par exemple) en présence d'air complétant la pression à 1 atmosphère afin de se trouver dans les conditions d'élargissement des raies rencontrées dans la pratique.

Ces mesures ont été faites pour treize polluants (éthylène, ammoniac, freons, monochlorure de vinyle, chloroprène, mercaptan, etc...)

Elles ont permis d'estimer les sensibilités qui, avec l'appareillage actuel, varient de quelques ppb/km (éthylène, ammoniac), à quelques centaines de ppb/km (mercaptan).

MESURES SUR LE TERRAIN

1- Choix du site

Il existe au Sud de Grenoble, dans la zone industrielle de Pont-de-Claix, sur une distance de 5 km, trois usines chimiques importantes A, B, C.

La pollution causée par ces usines sur le plateau de Champagnier situé à proximité, a été mesurée au cours des dernières années par les techniques classiques, par prélèvement. Pour un type de temps particulier (faible vent du sud), seuls les polluants issus de C arrivent en ce lieu ; il s'agit de butadiène, acétaldéhyde, chlorobutadiène, organiques chlorés, acide chlorhydrique, chlore, tertibutylparacrésol, chloroprène.

Ce dernier est détectable par notre appareil avec une sensibilité de 100 ppb, sur un trajet de 500 mètres. Nous avons donc surveillé l'apparition de chloroprène sur ce site pendant six mois.

2- Surveillance du chloroprène sur ce site

Avant de commencer cette surveillance, nous avons procédé à six essais en pollution artificielle de chloroprène, (tissus imprégnés de liquide disposés à proximité du faisceau) avec une mesure simultanée par prélèvement et chromatographie.

Le rapport des concentrations mesurées par les deux méthodes est :

$$\frac{C_1}{C_c} = 1,5 \pm 0,4$$

Ensuite, nous avons effectué 27 surveillances systématiques d'une durée de quelques heures chacune, dont 11 étaient couplées à des mesures par chromatographie après prélèvement.

24 d'entre elles ont indiqué une absence de chloroprène (sensibilité 100 ppb environ sur le parcours de 500 mètres). Il existe 10 de ces mesures doublées par la chromatographie, qui confirme le résultat.

Les trois autres surveillances ont montré des pics de pollution (quelques PPM pendant quelques minutes) une seule est couplée à une mesure chromatographique, qui confirme la mesure.

Malgré la présence de nombreux autres polluants, aucune interférence ne nous est apparue.

CONCLUSION

Nous pensons avoir montré la faisabilité de la télé-détection de polluants atmosphériques par la mesure de l'absorption différentielle de deux faisceaux délivrés par le laser à deux fréquences. Un important travail technologique reste à faire pour alléger l'appareil et le rendre banalisable. Un effort portant sur l'amélioration de l'électronique, la diminution du bruit et l'extraction du signal du bruit résiduel doit permettre de gagner un facteur dix sur les sensibilités. Il est possible d'étudier des appareils simplifiés et spécifiques d'un polluant (chlorure de vinyle, ozone, etc...) Il ne faut pas perdre de vue que cette méthode peut être utilisée avec d'autres sources que le laser à Co_2 (lasers à Co, HF, Co_2 isotopique, etc...)

Nous remercions les Communautés Economiques Européennes (Programme Recherche et Environnement) et le Commissariat à l'Energie Atomique (Délégation au Programme d'Intérêt Général) qui nous ont permis de mener à bien cette étude.

Contractors : 1) Laboratoire de Spectronomie moléculaire - Université Paris VI
2) Office National d'Etudes et Recherches Aéronautiques - Chatillon

Contracts n°: 1) 054-74-1 ENV F
2) 057-74-1 ENV F

Project leaders : 1) L. HENRY
2) A. GIRARD

Titles of projects : 1) High resolution absorption spectra of CO
(around 4600 and 2340 nm) NO (around 5200 nm)
and CH₄ (around 3300 nm)
2) High resolution absorption spectra of CH₄
(around 7700 nm) and SO₂ (around 9000 nm⁴)

The fundamental absorption band of NO and CO, the ν_3 band of SO₂, the ν_3 and ν_4 bands of ¹²CH₄ and ¹³CH₄ and the first overtone of NO and CO were spectroscopically analysed. The centres of the absorption lines, the relative intensities and the pressure broadening were measured for each line ; the accuracy for the wavelength of the lines varies from 3×10^{-7} to 8×10^{-6} according to the line intensity.

These spectra tables allow specific absorption measurements at various pressures because the broadening of the lines due to the pressure is a linear function of a coefficient also measured. For stratospheric measurements of these pollutant gases, the accuracy of the position of the lines is good enough.

For some other gases, in particular chlorinated compounds which may affect the ozone layer, it is intended to establish also absorption spectra of equivalent accuracy, to be used.

Contractor : Commissariat à l'Energie Atomique -
Centre d'Etudes Nucléaires Fontenay-aux-Roses (F)

Contract n° : 081-74-1 ENV F

Chef de projet : Mr. P. Zettwoog

Titre du projet : Etude des caractéristiques des rayonnements émis
pour les panaches des fumées industrielles

The STEPPA laboratory intended to develop a radiometer which could measure the light emitted by the hot gases at the exit of stacks to calculate the concentration of SO_2 (emission band at 8900 nm). As the temperature of the gases is not known, it is also necessary to calculate it by measuring the infra red emission of CO_2 (at 4300 nm) assuming it irradiates in the same way as a black body. Also the particles can irradiate light energy which is measured within two wavelength bands not absorbed by the atmosphere (3600 and 11 000 nm).

The radiometer, Pyro 4, has been built with these four bands filters and its performances checked in laboratory conditions and field conditions during the Lacq (1975) and Drax (1976) campaigns. When the conditions are constant, in laboratory, the assumptions give reasonable results.

In field conditions, it is necessary to perform successively four different measurements aiming at the plume itself then at the sky to correct from the background. Unfortunately, during the Lacq and Drax campaigns, the plumes were rapidly moving in and out of the field of view ; so the fluctuations of the observed SO_2 emissions and the plant emissions were not constantly correlated.

Improvements are still necessary to automatically reject the measured signals when the plume is no more in the field of view.

Introduction : INTEREST OF REMOTE SENSING

Optical remote sensing methods are able to measure the total amount of a pollutant in a vertical column overhead and, by carrying the instrument in a car, to map in a short time the dispersion of the pollutant over an emission site.

SO_2 and NO_2 can be detected by measuring their optical absorption, in the ultraviolet range for SO_2 and in the blue range for NO_2 .

The sunlight scattered by the atmosphere can be used as a source for the detection of these two pollutants.

PRINCIPLE OF THE SELECTIVE MODULATION RADIOMETER

The selective modulation radiometer developed at ONERA uses cells filled with the gas to be detected, to measure the absorption of this gas in the atmosphere. These cells act as the best possible filter and ensure a very high discrimination against other effects.

The light flux coming from the explored direction goes simultaneously through four cells containing different quantities of the gas to be detected, and is later measured by a detector (Fig. 1) : two cells are filled with the same quantity U_2 of the gas, one with a quantity U_3 greater than U_2 , and the fourth is empty or contains a gas which is not absorbing in the considered spectral range. A modulating disk (chopper) masks alternatively the first two cells, then the latter two.

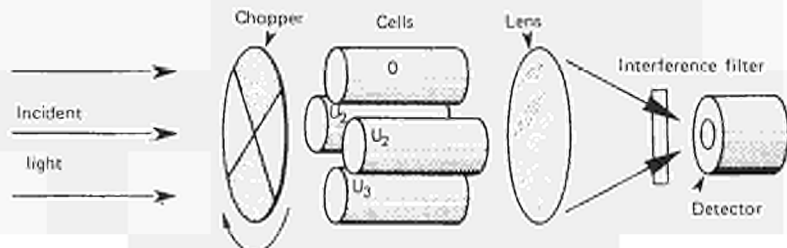


Fig. 1 - Schematic diagram of the four-cell radiometer.

In the absence of any pollution on the atmospheric path, the detector receives alternatively fluxes proportional to $2 \tau(U_2)$ and $1 + \tau(U_3)$ where $\tau(U_i)$ is the transmission of the cell filled with the quantity U_i integrated over the spectral range considered. If $U_i = 0$, $\tau(0) = 1$.

If U_2 and U_3 were chosen such that

$$2 \tau(U_2) = 1 + \tau(U_3), \quad (1)$$

the signal received by the detector is constant, its alternative component being null.

Then, when a small quantity ϵ of pollutant is present in the atmospheric path, the fluxes transmitted through the two groups of cells are non longer equal because integrated transmissions $\tau(U_i)$ are such that :

$$\tau(U_i + U_j) \neq \tau(U_i) \times \tau(U_j) ;$$

an alternative component of the signal results, which is proportional to ϵ and to the total flux received. The ratio of the alternative signal to the continuous signal is thus proportional to the quantity to be measured.

This ratio, or modulation rate, is measured by an automatic gain control amplifier.

Thus the absorption of an unknown quantity of gas is measured by comparing the variations of the transmissions induced by different quantities of the same gas.

PERFORMANCE

The limit of detection depends on the strength of the absorption band, on the luminance of the source and on the sensitivity of the detector.

In the case of NO_2 and SO_2 detection, the sensitivity is limited by the spectral fluctuations of the scattered skylight which are important in the presence of aerosols (cloudy sky).

A detection limit of 3 ppm. meter has been achieved with SO_2 and NO_2 with a blue cloudless sky as a source.

The main advantage of the four-cell radiometer over spectrometer or correlation spectrometer is its simplicity. It needs neither slits nor gratings, which limit considerably the flux admitted by the instrument. No spectral misalignment is possible, because the wavelengths at which the absorption is measured are always the absorption wavelengths of the gas itself.

Comparisons with results obtained by other teams using a correlation spectrometer have been made during the three last campaigns of remote sensing of pollution, organized by the Commission of the European Communities (Lacq, France, July 75 ; Brax, England, September 76 and Cordonnais, France, September 77). These comparisons demonstrate good agreement between the two types of instrument.

CONCLUSION

A new type of remote sensing instrument, the selective modulation radiometer, has been built and tested. Determination of total vertical amount of SO₂ and NO₂ with a detection limit of 3 ppm. meter has been achieved by using the light scattered by the atmosphere as a source.

This radiometer, with the same performance as other more complicated and expensive spectrometers, will be very useful as a cheap instrument for the study of the pollution problems.

PUBLICATIONS :

"Physics in Industry" Dublin 9-13 March 1976. Ed. E.O'Mongain and C.P. O'Toole, Pergamon Press.
Mesures-Régulation-Automatisme, vol. 42, No 7/8, juillet-août 1977, p. 39-46.

Contractor: C.N.R.-Laboratorio Inquinamento Atmosferico
Via Montorio Romano, 36 - 00131 Roma (ITALY)

Contract n^o: 068-74-ENV. I

Project Leader: A. Liberti, I. Allegrini

Title of project: Pollutants Evaluation by Means of Fluorescence

The aim of this work was two fold: I) Evaluation of Laser induced fluorescence as a tool for the Remote Sensing of selected gaseous components; II) Study of NO₂ and particles fluorescence in the view of their possible interferences when measuring other species by means of conventional Lidar Raman instruments.

I) Experimental

The exciting radiation has been provided by either a CW Argon Ion Laser or a N₂ pumped Frequency doubled Dye Laser. The excitation set up provides CW lines in the blue-green region of the spectrum with additional lines in the near UV, and pulsed beams from 600 down to 250 nm in the 10⁴W peak power.

Standard NO₂ atmospheres have been generated by means of permeation tubes which have been standardized at several temperatures.

Standard aerosol atmosphere were provided by a nebulizer whose design allows the generation of very small particles. Particles concentration is controlled by the air nebulizing flow rate, while particle size by the concentration of the solution. The chemical species can be anything which could be dissolved in a relatively low vapour pressure solvent like water, alcohol and benzene.

The characterization of the aerosol as to its size was performed "on-line" by means of a newly developed simple and reliable forward scattering photometer. Two pin photodiodes collect light scattered at 10 and 20 deg in the forward direction. An electronic circuit measures the ratio of the two signals which depends on particle size and is practically independent upon the refractive index. A detailed theoretical investigation on Mie equation applied to the instrument confirms that the instrument is effective in sizing particles between 0.1 and 2 μm . Experimental tests with a variety of particles, including standard latex spheres, were in agreement with the theory.

Most of experiments were carried out on 0.3 μm particle size on aerosols whose particle concentration was 10⁵ cm⁻³.

II) Results and discussion

Fluorescence spectrum of NO_2 obtained by a CW excitation, extends down to the far red region. The observed maximum at 600 nm is probably due to loss of efficiencies in PMT and monochromator. Distinct vibrational spikes were observed for irradiation at 488 nm, while the intensity of fluorescence decreases by a factor of two for irradiation at 514.5 nm.

Irradiation with pulsed laser at 426 nm provides a very intense fluorescence which reduces to 1/3 at 506 nm. Irradiation at 578 yields a not discernible counting rate.

The quenching factor in air has been found being about 10^{-5} at 488 nm which agrees with previously published values, thus a remote measurement of NO_2 can be performed with enough sensitivity if a pulsed laser oscillating at about 400-450 nm is used. The minimum detectable concentration increases to unpractical values if fluorescence from particles is taken into account.

The effect of NO_2 fluorescence on Raman return from gases has been evaluated experimentally by comparison of N_2 Raman scattering with fluorescence by NO_2 . Results show that the interference is very strong and might render impractical the Lidar scheme for ambient concentrations of most pollutants, limiting the usefulness of Lidar on stack measurements. The interference is zero if the irradiating wavelength is less than 380 or larger than 600 nm.

Most of measurements on particles have been obtained from polynuclear aromatic hydrocarbons which are present in atmosphere and are also found in high-boiling petroleum distillate (Tar). Spectra of aerosols do not differ from those obtained by pure compounds in solid form. All samples show fluorescence bands with superimposed Raman lines. As a general rule, fluorescence greatly increases at shorter irradiation wavelengths. Crysenes yields the most intense fluorescence spectrum, while tar shows only fluorescence which is vanishingly small for irradiation in the UV region.

CW irradiation at 488 nm 1W shows that the ratio of anthracene and tar fluorescence on N_2 Raman scattering is 10 and 3.5 respectively. Scaling this figure to the concentration of most pollutants in stack plumes, indicates that a few fluorescing particles cm^{-3} greatly reduces the S/N ratio for Raman Lidar.

A study on the evaluation of Laser Raman detection of particulates such as H_2SO_4 , show that a few particles cm^{-3} of quantum efficiency 0.1 increases the minimum detectable concentration to unrealistic values.

III) Conclusions

NO₂ can be detected by Laser fluorescence with depth resolution comparable to Raman, but interferences from particles greatly reduces the sensitivity. Fluorescence from aerosols makes unpractical the efforts to detect gaseous or particulate pollutants by laser Raman. Fluorescence effects can be reduced by a proper irradiating wavelenghts or by time-resolved spectrometry, but this is not feasible if the aerosol radiative lifetime is very small.

Such results would be properly extended if a better knowledge on Raman and fluorescence cross sections of particles of different sizes and informations on aerosol radiative lifetimes will be achieved in the next future.

IV) Papers produced

- 1) I. Allegrini, A. Cecinato, C.C. Gravatt, A. Napoli: "Simple and Rapid Particle Size Measurement by Means of Forward Light Scattering". Submitted to "J. of Colloid and Interfaces Science".
- 2) I. Allegrini, A. Cecinato, C.C. Gravatt, A. Napoli: "Physical Properties of Fine Particles by Means of Elastically Scattered Laser Radiations". Paper given at ECE-ONU Seminar on fine particles - Villach (1977).
- 3) I. Allegrini, N. Omenetto, L. Parma: "Aerosol Characterization by means of Laser Induced Raman and Fluorescence Scattering". Ibid.
- 4) I. Allegrini, P. Benetti, N. Omenetto: "In-Situ Chemical Evaluation of Particulate Matter by Means of Raman and Fluorescence Scattering". Submitted to "Environmental Science & Technology".

Contractor: Università di Pavia

Contract n° 077-74-10

Project leader: M. Omenetto

Title of Project: " RLMOTE SENSING OF GASES BY LASER INDUCED FLUORESCENCE".

1. Experimental set-up.

1.1. Laser source: Molelectron tunable dye laser pumped by a nitrogen laser (Models UV 400 and DL 200) provided with five KDP crystals for frequency doubling. Pulse characteristics: peak power 10-50 KW, 5 ns FWHM, 10 Hz repetition rate. Spectral bandwidth approximately 0.01 nm.

1.2. Fluorescence cells. Two fluorescence cells have been used: the first is a flowing system made out of monel internally coated with black teflon and the second one is made out of anodized aluminium. Both cells can also be used in a static system. Pure SO₂ from a tank and SO₂/air mixtures from a permeation tube were admitted in the cells.

1.3. Optics. The laser beam is focussed by a lens in the cell center. The exit fluorescence window at 90° with respect to the laser beam is directly coupled either to a high luminosity monochromator (Jarrell-Ash model 82463) or with a broad band filter or interference filter. Collection efficiency is not higher than 1%.

1.4. Electronics. Gated photon counting. Signal from PMT tube (Philips 56 TUVF) is fed into an amplifier/discriminator unit (Laben models 6150-6130) and read on a 50 MHz counter enabled to count for a variable gate time by a delayed gate generator unit driven by the laser pulse. A commercial unit (SSR Quantum Photometer, models 1140A and B) was also used. For lifetime measurements, a variable gate as given by a boxcar integrator (PAR 164) was slowly swept across the fluorescence waveform.

2. Results and discussion.

2.1. Fluorescence detection of SO₂ and NO₂.

The experiments were concentrated on the analytical performances of the fluorescence method when low concentrations of SO_2 and NO_2 are admitted in the fluorescence cell via a calibrated permeation tube. Delivery rates of the tubes were $4 \mu\text{g}/\text{min}$ for SO_2 and $0.2 \mu\text{g}/\text{min}$ for NO_2 , at 25°C . Sulphur dioxide, excited with the laser tuned in the region 260-300 nm shows a broad spectrum peaked around 320 nm. For NO_2 the laser was set at 421 nm and again a broad fluorescence spectrum at longer wavelength resulted. For SO_2 excited at 265 nm, a S/N ratio of 8 was obtained with a concentration of 18 ppm in air and therefore a S/N ratio of 2 would be given by approximately 5 ppm. For NO_2 , a S/N ratio of 2 would be given by 1 ppm. Measurements performed with a Joulemeter on the fundamental output frequency of the dye showed that our laser delivers approximately 300 microjoules which would correspond roughly to 60 KW for a 5 ns pulse. With a 10% conversion efficiency, 6 KW per pulse would be available for the excitation. Since saturation effects can safely be neglected, any increase in the laser power would linearly decrease the detectable amount of SO_2 and NO_2 . If this is coupled with an improved optical collection efficiency, the detectability for both gases can be pushed down well below the ppm level.

2.1. Aerosols fluorescence.

A possible interference in the backscattered signal from Lidar apparatus is due to the potential fluorescence of aerosols excited by the propagating laser beam. This fluorescence was found to interfere for NO_2 measurements but it is usually neglected in Lidar equations. The aim of our measurements was to characterize the spectrum of the aerosol fluorescence as well as to evaluate the lifetime of the process. Typical conditions for the measurements were: particle concentration in the cell 10^5 cm^{-3} , mean particle diameter $0.4 \mu\text{m}$, solvent cyclohexane. Tar and fluoranthene were the two compounds selected because it was felt that these substances were representative of two typical classes of aerosols likely to be found in polluted areas. The spectra obtained are shown in Figures 1 and 2. From these figures it is worth noting that i) tar spectrum extends

from the excitation wavelength to the red region of the spectrum, thereby overlapping the fluorescence of NO_2 and ii) fluoranthene fluorescence excited at 260 nm starts in the visible region of the spectrum with a broad maximum around 450-500 nm. Thus, fluoranthene fluorescence would not interfere with the SO_2 fluorescence which is peaked at 320 nm. However, tar will certainly interfere with the NO_2 measurements. Calculations based on the assumption of collecting fluorescence light from both the gases and the aerosols with the same spectral bandwidth show that the lifetime of the two processes is crucial for the evaluation of their mutual influence and therefore for the analytical feasibility of the method. Experimental results showed that both tar and fluoranthene lifetimes seem to be in the microseconds range. If this is the case, then when the aerosol load is not very heavy, temporal discrimination would be feasible between the two fluorescences. We strongly feel that it is essential to realize that the study of the potential feasibility of the fluorescence technique for Lidar applications cannot be separated by a parallel study of both spectral distribution and lifetime of the aerosol fluorescence.

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- 1) I. Allegrini, N. Omenetto and L. Parma, "Aerosol characterization by means of laser induced Raman and Fluorescence scatter" Proceedings of the Seminar on Fine Particulates, ECE-GNU, Villach (Austria), October 1977
- 2) I. Allegrini, P. Benetti and N. Omenetto, "In situ chemical evaluation of particulate matter by means of Raman and fluorescence scatter" submitted to Environmental Science and Technology.

Figure 1. Spectral distribution of laser excited fluorescence of tar aerosol. The spectrum is not corrected for the instrumental response.

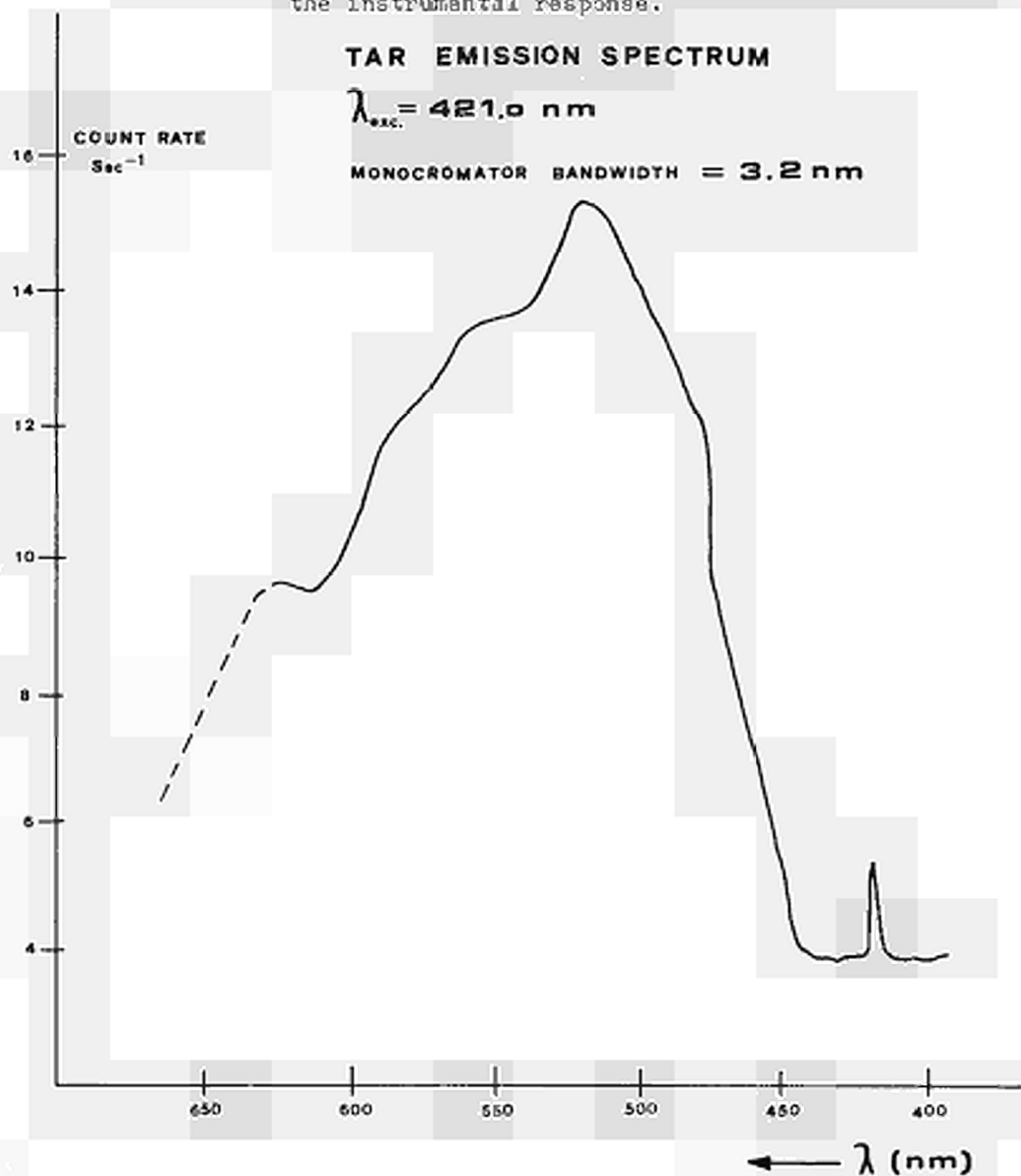


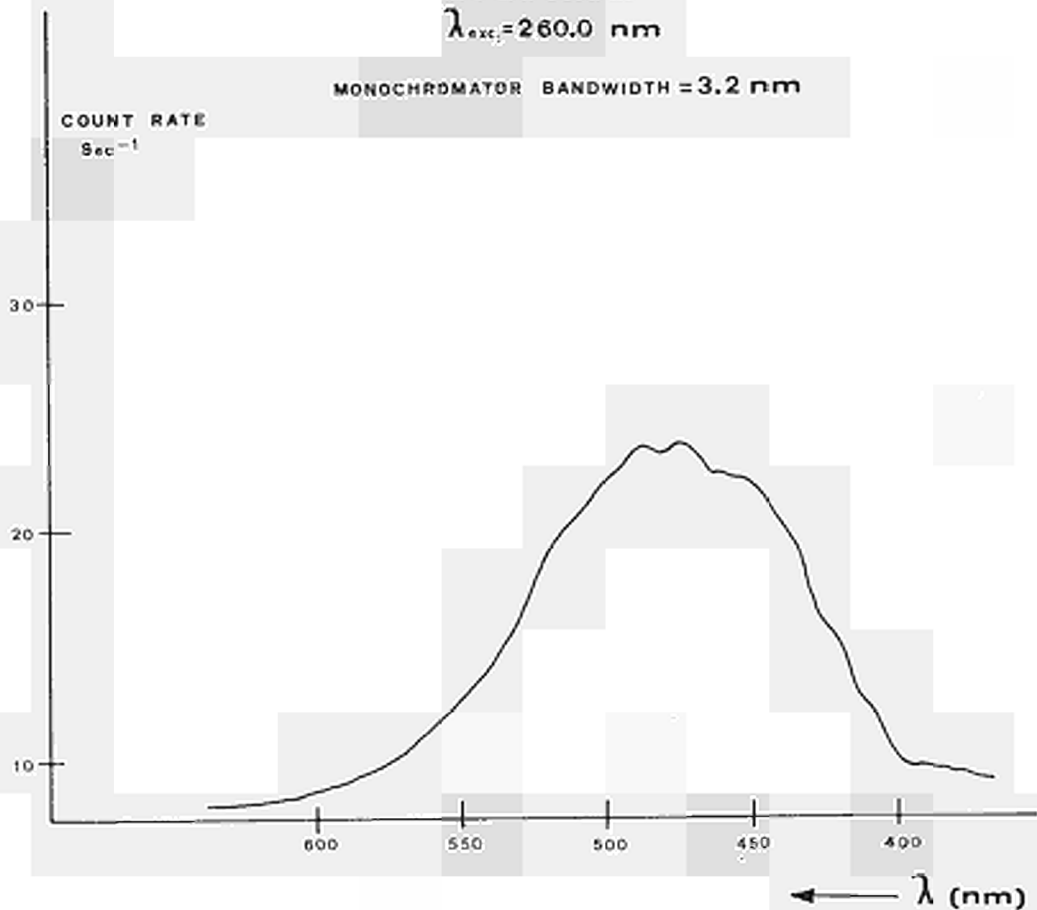
Figure 2. Spectral distribution of laser excited fluorescence of fluoranthene aerosol. The spectrum is not corrected for the instrumental response.

FLUORANTRENE EMISSION

SPECTRUM

$\lambda_{exc} = 260.0 \text{ nm}$

MONOCHROMATOR BANDWIDTH = 3.2 nm



Contractant : TECNECO S.p.A., S. Ippolito (Fano), Italy

N° du contrat : 083-74-77 ENV I

Chef du projet : OTTAVIO VITTORI (*)

Titre du projet : "MONITORING AIR POLLUTANTS FROM ISOLATED AND
LARGE SOURCES"

The research concerns field monitorings of SO₂ atmospheric patterns by a Teletec, an original version of Mask Correlation Spectrophotometer (Bonafé U. et al., 1976).

The campaigns had the general purpose of establishing the role of the Teletec in the field of atmospheric pollution problems.

Field experiments were performed at Ostiglia (Oct. 1975) and Ravenna (May 1976) in Italy, Lacq (July 1975) in France and Drax (Sept. 1976) in England. Several European research teams attended both the two latter campaigns, directly organized by C.E.C..

Teletec can operate with a light source of either artificial lamp or sky light. During the field experiments an improvement in its performance was reached: an electronic device was used in order to eliminate "scintillation" phenomena which had been noticed to pose limits on the geometrical distances that the instrument can cover (Evangelisti et al., 1977).

The part of the research that was concerned with long horizontal optical paths (artificial light source) gave the following results:

- 1) The optical depth measured along SO₂ plume cross sections, interpreted in terms of the Gaussian diffusion model, leads to the computation of actual vertical diffusion coefficients. The main parameter entering the model is the SO₂ optical depth along an axis perpendicular to that of the plume (Giovanelli et al., 1976).
- 2) The experimental data obtained during the various campaigns are consistent with both the meteorological parameters as measured in situ, and the intensities of the local SO₂ sources.
- 3) Measurements performed by other teams and concentration data as furnished by a network of point chemical sensors strengthen the validity of the Teletec performance (Vittori et al., 1977). Some examples of the significant aspects of the results obtained in the Ostiglia campaign are reported in Table 1.

Sky light measurements presents some uncertainty, as the light source is distributed along the path to be monitored, while the

(*) Collaborators : G. Giovanelli, T. Tirabassi (C.N.R., Bologna)
F. Gianni, E. Rossi Brunori (TECNECO S.p.A., S. Ippolito)

artificial source establishes unequivocally the path between the lamp and the instrument. Although Mask Correlation Spectrophotometer measurements with sky light have a qualitative character, they nevertheless give valuable information on the contaminant pattern.

In the above mentioned campaigns, the following sky light measurements were carried out:

- a) mass fluxes from an isolated source (Ravenna) (Tirabassi et al., 1976);
- b) vertical burden measurements (Ostiglia and Ravenna);
- c) plume parameters which could be used for reconstructing its patterns (Ostiglia) (Bacci et al., 1977).

The plume bidimensional reconstruction in terms of SO_2 optical depth was the most outstanding result obtained during the campaigns. The plume optical depths, as measured by Teletec from different zenithal and azimuthal angles, are given by simple geometrical projections to a direction normal both to the ground and to the wind direction. Owing to the qualitative character of the data, they have been normalized according to the actual SO_2 emission. The plume distributions are expressed in terms of optical depth isoplethes.

The computed plume height is in very good agreement with Briggs' formula (about 540 m for the two lower chimneys). The validity of the Briggs' formula in expressing plume heights had already been verified in the same area (Bacci et al., 1974). For the third chimney (220 m high), the calculated plume rise was about 700 m. Such a difference in behaviour of the three plumes may explain the shape of the optical depth isoplethes near the emission sources. At a distance greater than 2 km, the shape of the optical depth isoplethes satisfactorily fits the theoretical concentration distribution of a single plume. Fig. 2 also shows the atmospheric vertical thermal gradient, as measured at Milano-Linate airport. The stability profile appears to justify the observed plume pattern.

In conclusion, it appears that the results obtained from this activity are even more satisfying than expected. Although, they clearly need further checks, they show the wide range of application of the instrument and of the connected methodology. A proper series of measurements on a well projected instrumental network can permit a valuable reconstruction of the spatial and temporal configuration of large atmospheric plumes.

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(*) Published under Contract 083-74-77 ENV I

Table I - SO₂ concentrations (p.p.m.) measured by the network of chemical sensors and by the Mask Correlation spectrophotometer

Day - Oct 23 - 1975											
Hour	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-20 1
Station N° 15	.005	.018	.019	.018	.036	.028	.027	.023	.013	.011	.013
Station N° 16											
Station N° 17											
Station N° 18	.012	.014	.013	.036	.034	.035	.036	.021	.020	.018	.020
Station N° 19	.028	.025	.028	.050	.045	.045	.034	.023	.017	.023	.021
1 st station	.030	.023	.020	.023	.028	.030	.028	.024	.021	.025	
2 nd station											
3 rd station											
Wind direction (REVERE)	10°	360°	300°	300°	300°	300°	300°	300°	300°	290°	290°
Wind direction (Station N° 15)	360°	310°	280°	270°	270°	270°	270°	270°	270°	270°	270°
Wind velocity (m/sec) (REVERE)	1.0	2.0	3.8	4.0	4.5	5.0	3.0	3.5	3.0	4.0	4.5
Wind velocity (Station N° 15)	1.2	1.8	2.5	2.8	4.0	3.8	3.0	2.5	2.0	2.5	3.0
Teletec meas.	.027	.021	.019	.026	.031	.034		.021		.028	.028

Contractor: Ministry of Health and Environmental Protection/
National Institute for Public Health,
The Netherlands

Contract n^o 084-74-7 ENV N

Project leader: dr.L.Strackee and dr.D.Onderdelinden

Title of project: Remote sensing of atmospheric pollution with the aid of
correlation spectrometry

I. Introduction

Remote sensing of atmospheric pollutants can be performed by measuring the characteristic pollutant absorption bands superimposed upon the sky light spectrum. The use of ordinary absorption spectrometry to obtain information about the amount of pollutant integrated over the vertical direction ("gas burden") is based on the in this case rather doubtful assumption of a constant temporal sky light spectrum. The commercially available Barringer device for the measurement of NO₂ and SO₂ in the atmosphere tries to circumvent this difficulty by the use of a special kind of correlation spectrometry based on multiple slit systems. Although this measuring technique is rather successful remaining difficulties are base line shift and non linear response, probably due to changes in the spectral shape of the sky light. Therefore absolute measurements of the gas burden necessary for the study of long range transport of air pollutants are very troublesome with this hard-ware correlator. In order to study this problem more closely the hard-ware approach in the instrument mentioned above was replaced by a more flexible "soft-ware" approach with the aid of a spectrometer coupled on line with a small laboratory computer.

II. Equipment

The sky light from the zenith direction is thrown via a mirror into a 3.5 inch telescope situated in the laboratory (see fig.1). Calibrated NO₂ and SO₂ cells can be inserted in the light path between the telescope and a 0.50 m scanning photospectrometer. The photospectrometer is equipped with an absolute shaft encoder for the wavelength output, which together with the analogue signals $i_s(\lambda)$ and $i_r(\lambda)$ is fed into a CAMAC interface with the computer. This first signal $i_s(\lambda)$ measures the light intensity in a wave-

length interval of 0.2 nm, the second signal $i_r(\lambda)$ gives the integrated intensity over a wavelength region of about 10 nm round about the small slit wavelength. The signal $i_r(\lambda)$ accounts for the intensity variations of the sky light intensity. Remote control and data handling are done with the aid of a core resident program. Measured spectra of the sky light are stored on disc from which they can be read for further evaluation.

III. Measurements

Sky light spectra have been measured several times a day during a few months. The measurements were performed with and without NO_2/SO_2 cells in the light path. An example of such a measurement is given in fig.2. The two upper graphs in this figure reproduce the narrow slit signal in the wavelength interval from 305 to 315 nm, respectively with and without a calibration cell, containing $600 \text{ mg/m}^2 \text{ SO}_2$, in the light path. Below 305 nm the intensity of the sky light falls sharply due to absorption of the UV light by ozone in the upper layers of the atmosphere. The absorption spectrum of SO_2 , extending up to 315 nm, is completely masked by the structure of the sky light spectrum itself. Similar measurements were performed in the wavelength region from 400 to 450 nm, the region were an absorption band of NO_2 is situated.

IV. Numerical correlation

The first step in the calculation of the gas burden from a measured sky light spectrum is the removal of the slope in the spectrum. This is done with a first order digital filter. The remaining spectrum contains only the "fine structure" of the sky light spectrum and the "fine structure" of the absorption spectrum. If it supposed that the oscillatory structure of the sky light spectrum is due to absorption in the outer layers of the sun it will hardly change in time. This means that a further reduction of the measured spectrum can be obtained by division with the measured (and filtered) spectrum on a day with a pollutant free atmosphere. The logarithm of the remaining spectrum contains the absorption spectrum of the pollutant. An example of the result of such a manipulation is given in fig.2c. It is apparent from the figure that the spectral irregularities are removed to a large extend and the calculated spectrum closely approximates the absorption spectrum of SO_2 (graph d). By least square fitting of the latter spectrum to the spectrum given in graph c one calculates an observed gas

burden of SO_2 of 560 mg/m^2 , which agrees reasonably well with the gas burden of the calibration cell.

V. Results

The linearity of the instrument was found to be better than 10% for gas burden up to 1500 mg/m^2 for NO_2 and 1200 mg/m^2 for SO_2 . Zero level variations of the instrument under varying meteorological conditions were difficult to determine because they turned out to be smaller than the estimated gas burden variations in the rural area where the measurements were performed (Bilthoven). The zero level variations in summer time due to instrumental noise for a measuring time of 1 s per 0.1 nm were calculated to have a standard deviation of 10 mg/m^2 for SO_2 and 2 mg/m^2 for NO_2 . For a mixing height of 1000 m this corresponds to a mean concentration of a few $\mu\text{g/m}^3$.

VI. Discussion

The principle aim of the study reported here was to demonstrate the feasibility of a correlation spectrometer for the remote sensing of air pollution, based on digital data retrieval of the sky light spectrum in the absorption region of the pollutant concerned. This approach allows a flexible method to correct for spectral variations of the light source used, either the natural sky light or an auxiliary lamp.

The main disadvantage of the instrument in its present form is the low light collecting efficiency. This difficulty can be removed when the spectrum is recorded quasi simultaneously with the aid of a television camera. Work along this line is in progress.

The work is carried out under contract n^o 084-74-7 ENV N of the E.C. Environmental Research Programme.

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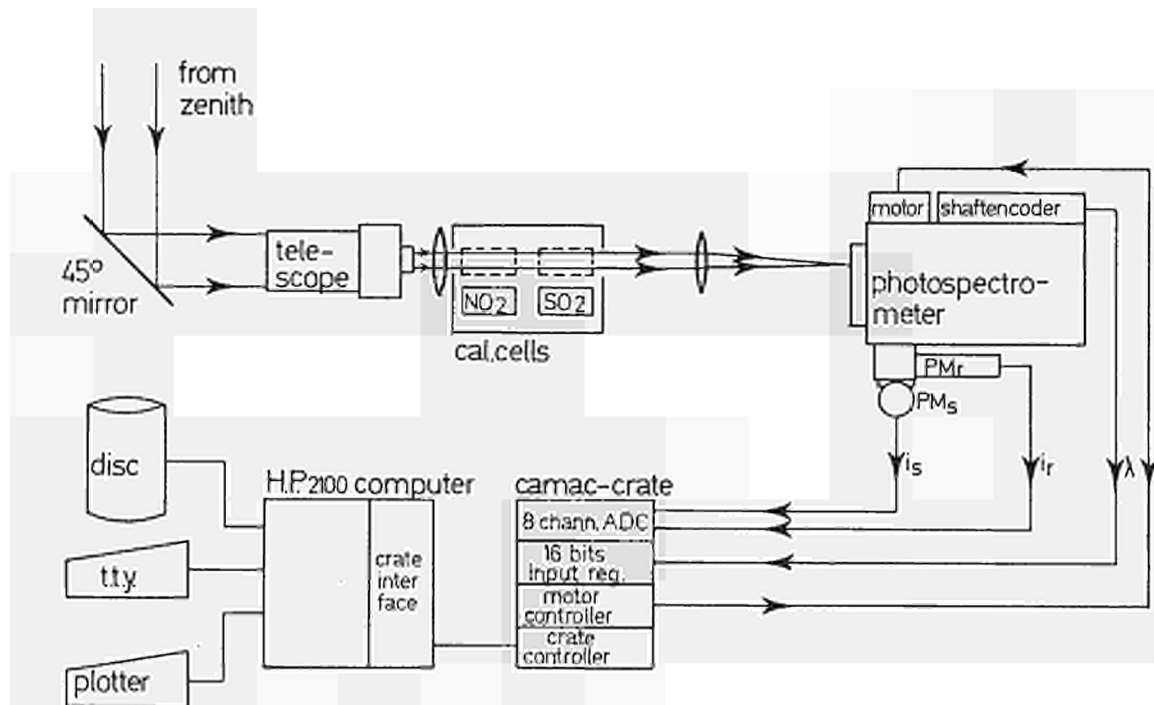


Fig. 1

The sky light collecting equipment and the data handling system.

- a. The sky light spectrum as measured with the narrow slit (0.2 nm).
 b. The same spectrum with a SO_2 -cell inserted in the light path.
 c. The logarithm of the ratio of the two upper spectra after division by the monitor signal i_p and numerical filtering of the "slow" components.
 d. The reduced absorption spectrum of SO_2 .

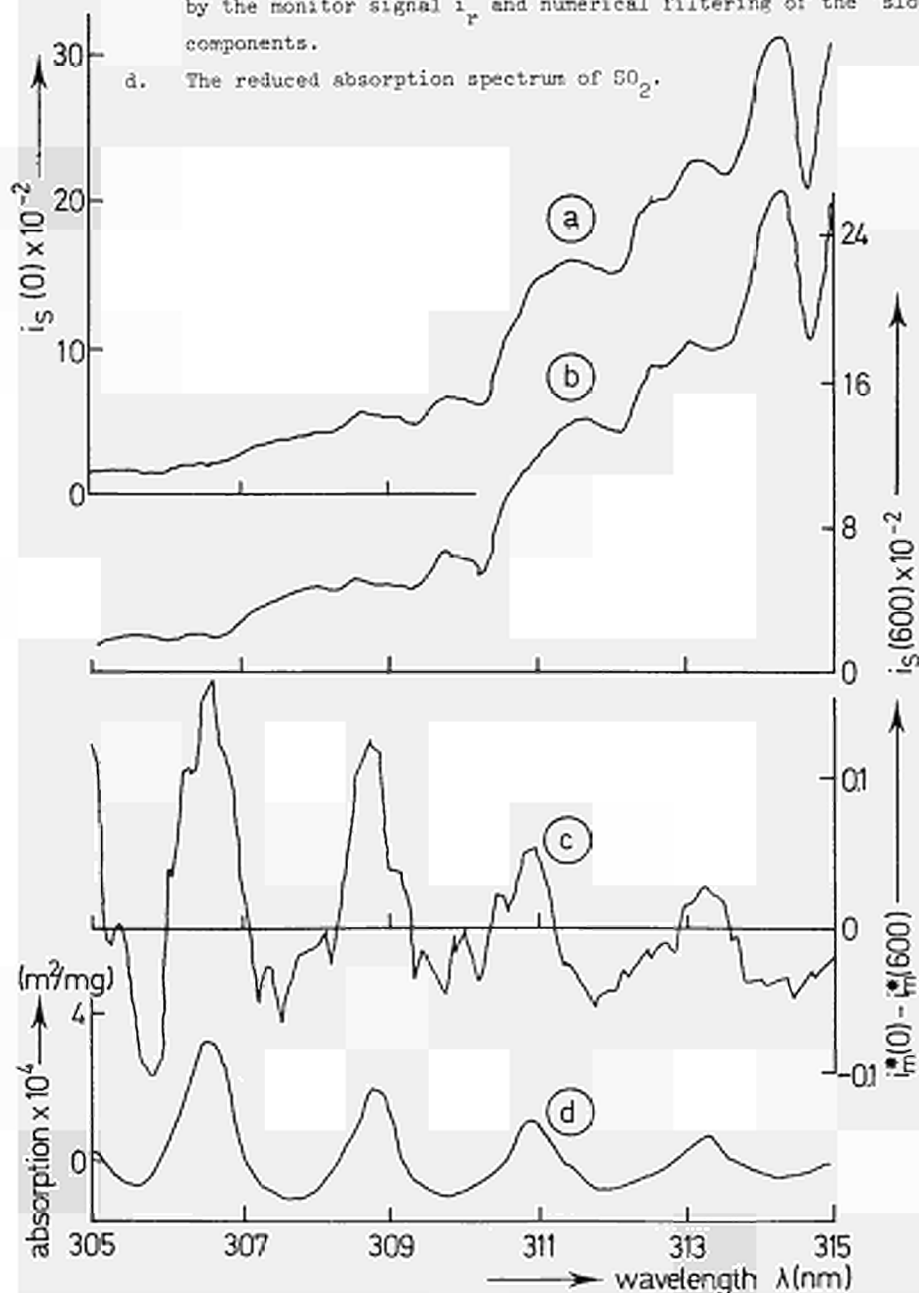


Fig. 2

Contractor : Institute of Applied Physics TNO-TH
Stieltjesweg 1, Delft
Contract n^o 107-74-11 ENV N

Project leader : Ir. H.J. Raterink

Title of project : Investigation of remote heterodyne detection techniques to measure air pollutants

In heterodyne detection radiation from an object is mixed on a detector with radiation from a coherent local oscillator, which is in general a tunable laser. The output signal of the detector with amplifier is detected at the difference frequencies between the two radiations, within the bandwidth of the detector system. This electrical bandwidth is in practice about 1500 MHz and can be scanned by one frequency channel or by an electrical filterbank, with which simultaneous registration of a number of frequency channels can be obtained. Main features of heterodyne detection are: a. a high spectral resolution (10^{-4} - 10^{-5} cm⁻¹ for 10,6 μ m) b. a high sensitivity (min. detectable power about 10^{-9} W/Hz) and c. a high angle resolution (small field of view).

Heterodyne detection has already been applied successfully in astronomy, per example to determine the presence of CO₂ in the Martian atmosphere. The goal of this research project was to carry out in two phases a feasibility study, concerning the application of remote heterodyne detection techniques for the detection and measurement of air pollutants. In Phase 1 of the research project a "state of the art" study has been carried out with respect to existing literature and to tunable lasers. Furthermore emission spectra of gases of interest have been selected, which were acceptable to the wavelengths of the available tunable CO₂-laser.

In addition an experimental set-up has been built, using components made available by the Astronomical Institute of the University of Utrecht. A variable path absorption cell has also been developed to measure absorption profiles. The experimental set-up is schematically shown in figure 1. A Sylvania tunable CO₂-laser, in which a sealed tube is applied, is used as local oscillator. A liquid nitrogen cooled Hg Cd Te photodiode is used as detector. For making spectral scans, the amplified signal of the detector is led to an electronic mixer, which local oscillator is fed by a sweeposcillator with a frequency range from 100 kHz to 1,4 GHz in the three bands.

The mixer output is followed by a 10 MHz low pass filter, an amplifier and a quadratic detector. With this system the passband of 10 MHz is located at both sides of the frequency of the sweeposcillator. An effective receiving band of 20 MHz is obtained, which corresponds with a spectral resolution of $1,5 \times 10^6$.

In order to obtain additional tunable wavelengths, the development of a flowing-gas CO₂-laser has been started.

In Phase 2 of the research study measurements were carried out on the sample gases CO₂, C₂ H₄ and NH₃ for the P22 and R16 laser transitions of the 00^o1 - 02^o0 vibrational band at 9,569 μ m and 9,294 μ m respectively.

The pressure and temperature of these gases in the absorption cell have been varied and N_2 has been added up to atmospheric pressure. The pathlength in the absorption cell was adjusted to 3,54 m. In order to obtain an analytical expression for the measured line profile a least squares regression of either a Doppler -or a pressure- broadened Lorentz profile according to theoretical formulas, was applied to a number of data points around the line center. From these analytical expression the line parameters as linewidth and absorption coefficients have been calculated. Absorption profiles for CO_2 and C_2H_4 are presented in figure 2 and figure 3 respectively as an example. From the noise level of the relevant measurements, the following sensitivities were actually achieved for a pathlength of 10 m:

for carbondioxyde	: 100 mg/m^3
for ammonia	: 4,0 mg/m^3
for ethylene	: 15 mg/m^3

By improving the signal-to-noise ratio of the detection system these sensitivities can be improved.

Publications

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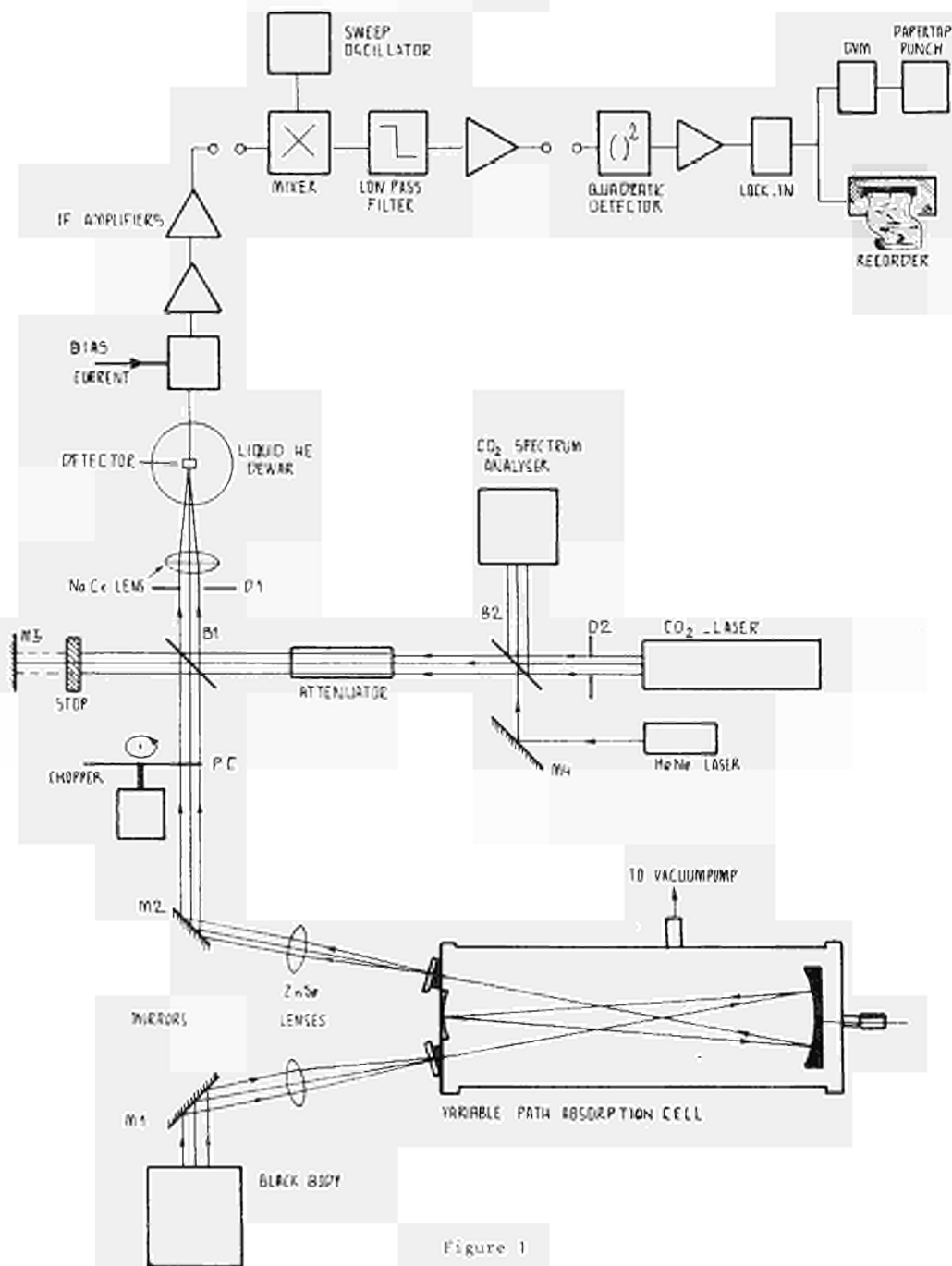
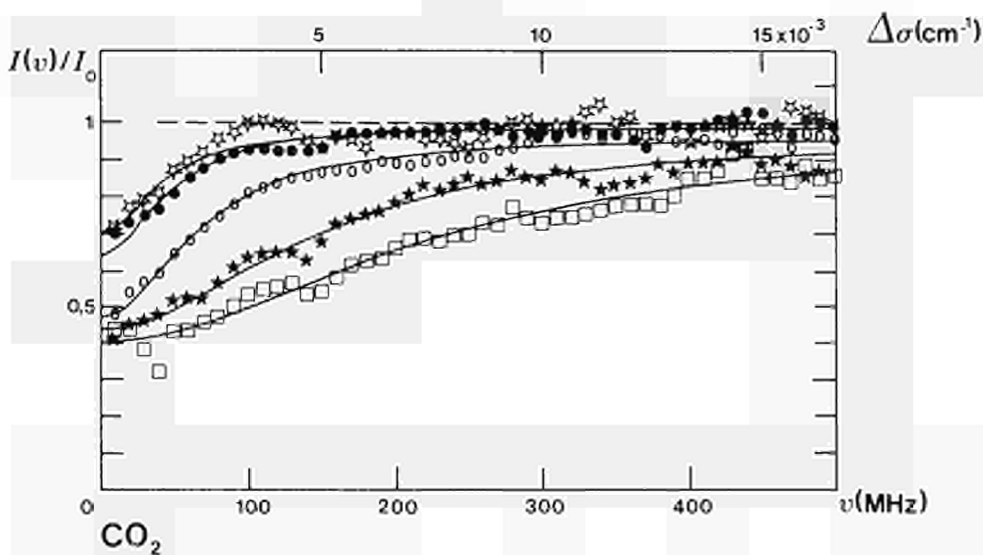
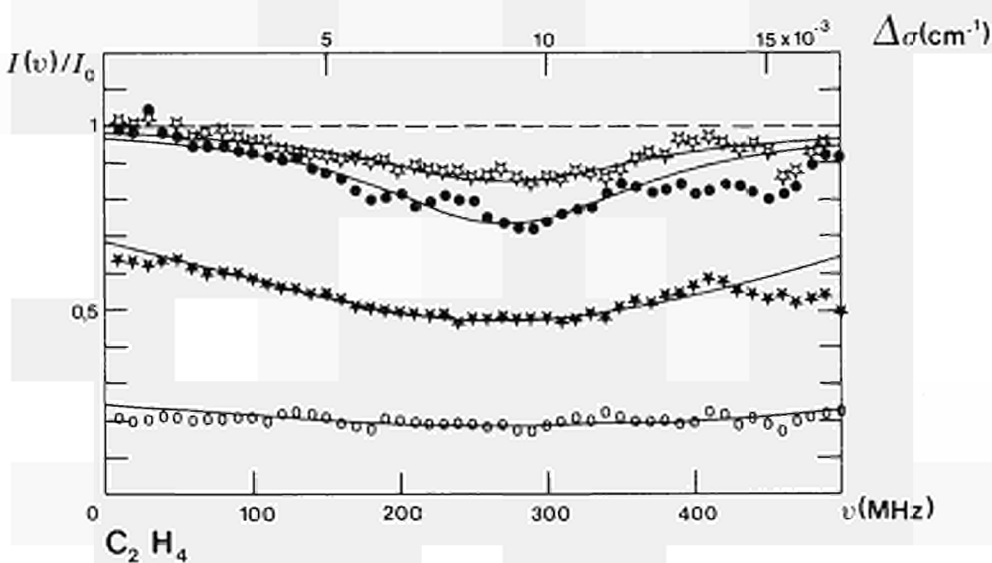


Figure 1



- ☆ : 290 Pa (2,2 Torr)
- : 570 Pa (4,3 Torr)
- * : 1430 Pa (10,7 Torr)
- : 2876 Pa (21,5 Torr)
- : 5730 Pa (43,0 Torr)

Figure 2



- ☆ : 290 Pa (2,15 Torr)
- : 570 Pa (4,3 Torr)
- * : 1430 Pa (10,7 Torr)
- : 2870 Pa (21,5 Torr)

Figure 3

Contractor: Department of Applied Physics, University of Hull

Contract No. O27-74-1 ENV.UK

Project leader: Dr. E.L. Thomas

Title of project: The remote sensing of chemical pollution in the atmosphere.

The original aim of the contract was to evaluate resonance Raman scattering as a means of remotely detecting atmospheric pollutants. Our initial work was restricted to those pollutants which had electron absorption bands within the tuning range of dye lasers or their second harmonics, viz. NO_2 , SO_2 and O_3 . Most of the work was concerned with NO_2 . Calculations, using assumed values for the resonance Raman scattering cross-sections, showed that the sensitivity of the technique would be insufficient for day-time use. During the day, the backscattered signal would be dominated by solar radiation. At night, the computer model predicted an NO_2 sensitivity of up to 10mg per cubic metre at ranges of up to six hundred metres.

Early on in the investigation, Rosen, Robrish and Chamberlin(1) reported experimental values for the resonance Raman scattering cross-sections of SO_2 and NO_2 which were several orders of magnitude larger than the values we had used in our models. Attempts were made to repeat the work of Rosen, Robrish and Chamberlin. However, at no time were we able to detect any Raman scattering which exceeded the strong fluorescence background intensity.

During the course of our early work on resonance Raman scattering, several authors(2,3,4) showed that differential absorption and scattering (DAS) was the most sensitive remote sensing technique for monitoring atmospheric pollutants. From this point on, efforts were concentrated on the differential absorption and scattering technique and an experiment was assembled to detect atmospheric nitrogen dioxide. The equipment consisted of a nitrogen laser pumped dye laser operating in the blue region of the spectrum (0.1mJ in a 10 nanosecond pulse at up to 500Hz), a 90cm parabolic receiving telescope, a half metre grating spectrometer and a sensitive, high gain photomultiplier with good noise characteristics. The output of the photomultiplier was fed to a transient digitiser and the return signals were accumulated in a minicomputer. Provision was also made for photon counting when the return signals were weak.

Due to data processing limitations, the maximum repetition rate of the laser was restricted to 100Hz. The equipment is shown in Figure 1.

When the laser parameters had been optimised, the absorption spectrum of atmospheric nitrogen dioxide was measured by detecting the return signals from a retro-reflector mounted on a building 233 metres away. Towards the end of the contract period, range resolved measurements were made whilst the laser was aligned on the retro-reflector. This was achieved by using photon counting equipment in the box-car mode with a minimum gate width of 50 nano-seconds.

In parallel with the work on a visible OAS system, a design study was carried out on an infrared DAS system. It was shown that many of the limitations of a visible DAS system could be overcome by operating in the infrared. Although the 'ideal' laser for an infrared DAS system does not exist at present, we believe that a system based on a high pressure, CO_2 laser transmitter and a heterodyne receiver would be a good compromise solution and would have the following advantages over a visible DAS system:

(a) An infrared DAS system would have a comparable range, range resolution and sensitivity to a visible DAS system.

(b) Several pollutants (e.g. O_3 , C_2H_4 , NH_3 , SO_2 etc) can be monitored using the same infrared equipment.

(c) The transmission of the atmosphere in the 8-14 micron window is always better than in visible region - especially under adverse weather conditions.

(d) Due to the extreme spatial and spectral filtering of a heterodyne receiver, background noise is not a problem and the system can be operated in daylight without a monochromator.

(e) An infrared system cannot cause retinal damage, and since the safe exposure levels are about 10^4 times higher than in the visible, an infrared system could be safely operated in populated areas.

The proposed layout of an infrared DAS system is shown in Figure 2. Work has already started to test the feasibility of such a system. A frequency stabilised hybrid TEA, CO_2 laser is being used as the transmitter and a frequency stabilised CW, CO_2 laser is being used as the local oscillator in the heterodyne receiver.

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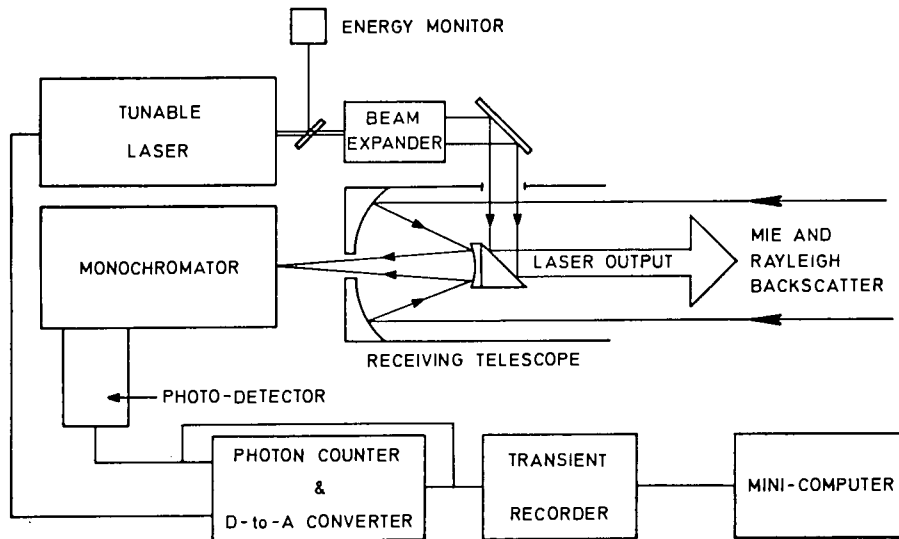


Figure 1: Pollution monitoring using differential absorption and scattering (DAS).

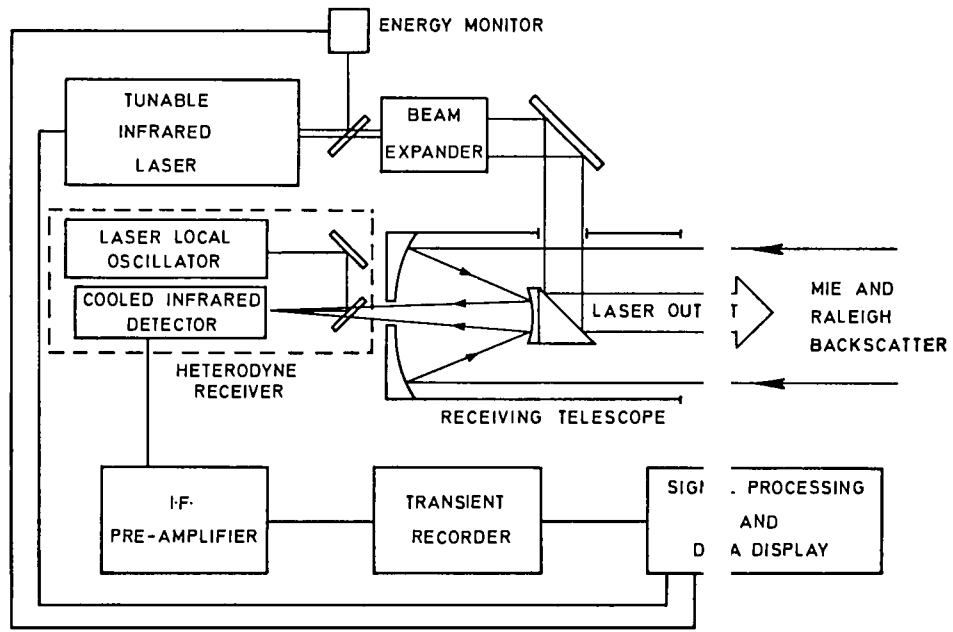


Figure 2.: Pollution monitoring using an infrared DAS system with heterodyne detection.

TOPIC 6 : ESTABLISHMENT OF A DATA BANK ON ENVIRONMENTAL CHEMICALS

Contractor: Technische Universität München
Institut für organische Chemie

Contract no.: 115-74-10-ENV-D

Project leader: Professor Dr. Ivar K. Ugi

Title of project: Coding of chemical Structures, Retrieval
of Substructures and Prediction of
Successors of chemical Substances in
the environment

In the present pilot study the applicability of a mathematical model of constitutional chemistry in the development of program systems for environmental studies was explored. In this model, chemical structures are represented by atom vectors and bond-electron-("BE-") matrices. Chemical structures, in this context, are individual molecules, or ensembles of molecules ("EM"), e.g. the partners of a reaction. Reactions are defined as isomerizations of an EM, and are represented by reaction-("R-") matrices. Addition of an R-matrix to the BE-matrix of an EM yields the reaction products of this EM.

A program system was developed with the following features:

1. A predictor module accepts a molecular structure ("problem structure") as input. Such a problem structure would typically be the molecular formula of a substance, whose chemical behaviour in the environment is to be studied. The environment is defined by the set of possible reaction partners occurring in this environment. The user may select these partners from a list contained in the program. Reaction operators and selection rules are applied to the problem structure to generate possible reaction products in

the given environment. The reaction products are separated into individual molecules, and collected in a data set for graphical output, or for further processing by other programs, e.g. a retrieval system.

2. An interactive retrieval system provides fast access to chemical structures and substructures that are stored on an external random access medium, typically a moving-head disc. The retrieval system operates on a hierarchically organized file of substructures, which is generated by a newly developed fragmentation algorithm. According to the rules of the mathematical model, fragmentation is defined as a successive lowering of the values of entries in the BE-matrix. In storage, each fragment has a pointer to each structure from which it was generated, and vice versa. Molecules have pointers to all first level substructures. Each level of substructures becomes the origin of a further level. Hierarchical organisation by means of a canonisation routine, storage saving data compression and fast addressing algorithms provide for the economy and minimal access times that are indispensable in an interactive system.

Operating characteristics of large computer systems, such as an IBM System /360-91, and "mini" computers (Digital Equipment PDP-11/45) were investigated in the above applications. The small system was found most suitable for development and operation of the retrieval system, when data bases are of reasonable size. Graphical input and output is most favorably handled, and access to peripheral devices is fast and easy. Programming effort is somewhat larger due to limited support of problem oriented languages, especially, when bit operations are involved, and due to a smaller set of debugging aids. Up to now, data are transported on storage media, because the larger system did not support direct coupling of the two machines.

Contractor: THE FRANKLIN INSTITUT , MUNICH

Contract No.: 126-65-4 ENV D

Project Leader: Paul N. Craig, Ph.D.

Title of Project: Preparation of Abstracts of Chemical Compounds in the Areas of Environmental Distribution and Transformation and Toxicology for Input into the ECDIN Data Bank

The Franklin Institute GmbH (FI GmbH) searched the literature to obtain data in the areas of environmental distribution and transformation and toxicology for more than 300 chemical Compounds. Condensates (abstracts) were provided on magnetic tape for 265 compounds for direct input into the ECDIN data bank.

Information was sought and data were acquired on the following categories.

- | | |
|---|---|
| 1. Environmental synthesis | 10. Effects on aquatic organisms |
| 2. Dispersion pathways | 11. Effects on reproduction, and teratogenicity |
| 3. Abiotic degradation | 12. Carcinogenicity |
| 4. Biological absorption, metabolism, and excretion | 13. Mutagenicity |
| 5. Biological retention and accumulation | 14. Allergic and immunological reactions |
| 6. Monitoring of environmental samples | 15. Phytotoxicity |
| 7. Intake by man and animals | 16. Effects on ecosystem |
| 8. Effects on humans | 17. Effects on inanimate objects |
| 9. Effects on animals | 18. Analytical methods |

The data for each chemical were evaluated according to specified guidelines and the condensates which were prepared on above mentioned categories were indexed and subjected to expert review. The final data were transcribed on 9-track computer magnetic tapes for input to the ECDIN data bank at Ispra. The 265 chemicals for which condensates were completed in this project are:

1. Acenaphthene
2. Acenaphthylene
3. Acetic acid
4. Acetic anhydride
5. Acetone
6. Acetophenone
7. 2-Acetylthiophene
8. Acrylonitrile
9. Adipic acid
10. Aldrin
11. Allyl alcohol
12. Allyl chloride
13. 2-Aminoethanol
14. m-Aminophenol
15. o-Aminophenol
16. p-Aminophenol
17. Aniline
18. Anthracene
19. Anthraquinone
20. Arasan
21. Azoxybenzene
22. 1,2-Benzanthracene
23. Benzene
24. Benzenethiol
25. Benzidine
26. 3,4-Benzofluoranthene
27. 10,11-Benzofluoranthene
28. 11,12-Benzofluoranthene
29. Benzofuran
30. Benzoic acid
31. Benzo(A)pyrene
32. Benzothiazole
33. 1,12-Benzopyrene
34. Bis-chloroethyl ether
35. Bis-chloromethyl ether (BCME)
36. Bromodichloromethane
37. Bromoform
38. 1,3-Butadiene
39. Butanol
40. 2-Butanone
41. Butylated hydroxyanisole
42. Butyl benzoate
43. Butyl cellosolve
44. N-Butylisothiocyanate
45. 2-t-Butyl-4-methylphenol
46. Butyraldehyde
47. n-Butyric acid
48. Cadmium sulphate
49. Carbaryl
50. Carbazole
51. Carbon tetrachloride
52. Chloral hydrate
53. Chloradane
54. Chloroacetic acid
55. m-Chloroaniline
56. Chlorobenzene
57. m-Chlorobenzoic acid
58. Chlorodibromomethane
59. 4-Chloro-1,2-dinitrobenzene
60. Chloroform
61. 3-Chloro-4-hydroxybenzoic acid
62. 5-Chloro-2-hydroxybenzoic acid
63. p-Chloromandelic acid
64. Chloromethylmercury
65. 1-Chloro-2-nitrobenzene
66. m-Chlorophenol
67. o-Chlorophenol
68. p-Chlorophenol
69. (4-Chlorophenyl)-acetic acid
70. 4-Chlororesorcinol
71. 5-Chlorouracil
72. 8-Chloroxanthine
73. Chlorpyrifos
74. m-Cresol
75. Cyclohexane
76. Cyclohexanol
77. Cyclohexanone
78. Cyclopentadiene
79. p-Cymene
80. DDT
81. Diallyl
82. 4,4'-Diaminodicyclohexylmethane
83. 1,6-Diaminohexane
84. Diazinon
85. Dibenzofuran
86. 1,2-Dibromoethane
87. 2,3-Dibromo-1-propanol
88. Dibutyltin salts
89. o-Dichlorobenzene
90. 1,1-Dichloroethene
91. 1,2-Dichloroethene
92. 2,4-Dichlorophenoxyacetic acid (2,4-D)
93. Dieldrin
94. Diethylamine
95. N,N-Diethylformamide
96. 2,5-Diethylthiophene
97. Dimethyldisulphide
98. 2,6-Dimethylnaphthalene
99. 2,6-Dimethylphenol
100. 3-4-Dimethylphenol
101. Dimethyl sulphate
102. Dimethyl sulphone
103. 1,7-Dimethylxanthine
104. 2,6-Dinitrotoluene
105. 3,4-Dinitrotoluene

106. Dioxane
107. Diphenyl sulphone
108. Disulfoton
109. p-Dithiane
110. Diuron
111. Endrin
112. Ethanol
113. Ethyl carbamate
114. Ethyl chloride
115. Ethylene glycol
116. 2-Ethylhexanol
117. Ethyl isothiocyanate
118. Ethylmercury chloride
119. Ethyl orthoformate
120. m-Ethylphenol
121. o-Ethylphenol
122. p-Ethylphenol
123. Fenac
124. Fenchlorphos
125. Fluorene
126. Formaldehyde
127. Formothion
128. 2-Formylthiophene
129. Freon
130. 2-Furaldehyde
131. Furfuryl alcohol
132. L-Glutamic acid
133. Guaiacol
134. Heptachlor
135. Heptachlor epoxide
136. Heptadecanoic acid
137. Hexachlorobenzene
138. Hexachloro-1,3-Butadiene
139. Hexachlorocyclohexane (mixture)
140. Hexachlorocyclopentadiene
141. Hexachloroethane
142. n-Hexane
143. 1-Hexanol
144. p-Hydroxyacetophenone
145. p-Hydroxybenzaldehyde
146. 3-Hydroxybenzoic acid
147. 2-Hydroxybenzyl alcohol
148. 4-Hydroxyphenylacetic acid
149. p-Hydroxythiophenol
150. Isobutyraldehyde
151. Isocyanic acid
152. Isodrin
153. Indene
154. Lauric acid
155. Lead salts
156. Lindane
157. Linoleic acid
158. 2,5-Lutidine
159. Malathion
160. Malonic acid
161. Mandelic acid
162. Mecoprop
163. Mercaptobenzothiazole
164. Mercuric chloride
165. Mercury
166. Metaldehyde
167. Methoxychlor
168. Methoxyethylmercury chloride
169. o-Methylbiphenyl
170. p-Methyl-N-butylbenzamide
171. Methyl chloride
172. 2-Methyl-4-chlorophenoxybutyric acid (MCPB)
173. Methylene chloride
174. 3-Methylindene
175. Methyl methacrylate
176. 1-Methylnaphthalene
177. 2-Methylnaphthalene
178. 2-Methylnaphthalene
179. 2-Methylthiobenzothiazole
180. Methyl trisulphide
181. 1-Methylxanthine
182. 3-Methylxanthine
183. Naphthalene
184. 1-Naphthol
185. 2-Naphthol
186. 1-(1-Naphthyl)-2-thiourea
187. m-Nitroaniline
188. 2-Nitroanisole
189. Nitrobenzene
190. 2-Nitro-p-cresol
191. o-Nitrophenol
192. N-Nitrosodiethylamine
193. m-Nitrotoluene
194. o-Nitrotoluene
195. 2-Nitro-m-xylene
196. NTA
197. Nuvan
198. 1-Octanol
199. n-Octylmercaptan
200. Oleic acid
201. Oxalic acid
202. Palmitic acid
203. Paraquat
204. Parathion
205. Pelargonic acid
206. Pentachloroethane
207. Pentachlorophenol
208. Pentadecanoic acid
209. Phenanthrene
210. Phenol

- | | |
|--------------------------------------|----------------------------------|
| 211. Phenylacetic acid | 238. m-Toluidine |
| 212. 2,3-(o-Phenylene)-pyrene | 239. o-Toluidine |
| 213. 1-Phenylethanol | 240. p-Toluidine |
| 214. Phenylmercury acetate | 241. 2,4,5-Trichloroanisole |
| 215. N-Phenylphthalimide | 242. 1,2,4-Trichlorobenzene |
| 216. 1-Phenyl-2-thiopropene | 243. 1,1,1-Trichloroethane |
| 217. Phloroglucinol | 244. Trichloroethylene |
| 218. Phthalic acid | 245. Trichlorophenol |
| 219. Propham | 246. Tricyclohexyltin hydroxide |
| 220. Propoxur | 247. Triethylphosphate |
| 221. Propyl gallate | 248. Triethylurea |
| 222. Propyl nitrate | 249. Triphenyltin salts |
| 223. 4-n-Propylphenol | 250. Tris(2-ethylhexyl)phosphate |
| 224. Pseudocumene | 251. Undecane |
| 225. Pyridine | 252. Undecanoic acid |
| 226. Rogor | 253. Uracil |
| 227. Sodium dodecylbenzenesulphonate | 254. n-Valeric acid |
| 228. 2,4,5-T | 255. Vanillin |
| 229. o-Terphenyl | 256. Vertraldehyde |
| 230. 1,2,4,5-Tetrachlorobenzene | 257. Veratrol |
| 231. 1,1,2,2-Tetrachloroethane | 258. Vinyl Acetate |
| 232. Tetraethyl lead | 259. Vinyl chloride |
| 233. Tetrahydrofuran | 260. Warfarin |
| 234. Tetrahydrofurfuryl alcohol | 261. Xylene |
| 235. Tetramethyl lead | 262. 2,3-Xylenol |
| 236. 2,2'-Thiodiethanol | 263. 2,4-Xylenol |
| 237. Toluene | 264. 2,5-Xylenol |
| | 265. 3,5-Xylenol |

The information input for these 265 chemicals resides in the ECDIN data bank at Ispra.

Contractants : UNIVERSITE PARIS VII
CENTRE D'ETUDES ET DE RECHERCHES DE TOULOUSE (CERT)
DE L'OFFICE NATIONAL D'ETUDES ET DE RECHERCHE
AEROSPATIALES (ONERA)

Numéros des contrats : 072.74.1 ENV F et
053.74.1 ENV F

Chef du projet : Professeur Jacques-Emile DUBOIS

Titre du projet : Pilote d'Information Structurale et de Comportement
INTégré et Évolutif (PISCINE)

Le projet PISCINE est une contribution française au projet Environmental Chemical Data and Information Network (ECDIN) ; il repose sur des développements complémentaires de deux systèmes français et leur expérimentation conjointe sur les fonds de données rassemblées pour le projet ECDIN :

- *le système DARC* (Université PARIS VII) : Description des structures chimiques, Acquisition, Restitution, Conception, pour le signalement topologique des composés chimiques,
- *le système SYNTAX* (ONERA-CERT) : Système d'Interrogation EXpérimental pour l'exploitation dynamique des informations et des données associées à ces structures.

L'objectif de ce projet complexe était de proposer des solutions informatiques aux problèmes généraux des banques de données et d'en démontrer la faisabilité et l'intérêt pour l'exploitation conversationnelle d'une banque de données chimiques, du type ECDIN.

Les travaux effectués, tant à PARIS qu'à TOULOUSE, en liaison étroite avec les responsables du projet pilote communautaire, ont fait la preuve que les

deux systèmes français apportaient des solutions avancées dans deux domaines :

- *l'intégration aisée*, dans les fichiers, de données (structures et autres), d'origines diverses et de formes variées, codées ou non,
- *l'accès conversationnel* aux données (restitution, édition), dans un LANGAGE NATUREL, familier aux utilisateurs.

Nous exposons ci-après les contributions spécifiques de chacun des deux systèmes.

1 - SYSTEME DARC

Pour la création de fichiers structuraux (formules développées codées dans une représentation topologique, biunivoque et non ambiguë), le système DARC propose deux méthodes :

- *le transcodage*, adapté à l'acquisition en DARC de masses de données structurales, déjà saisies et codées en code topologique (CAS - RIII/SDF, par exemple) ou en Wisswässer Line Notation.
- *la saisie assistée* à l'aide d'un appareil spécialisé "Topocodeur" et le codage automatique ; cette méthode convient parfaitement à l'acquisition des structures pour une banque de données de quelques dizaines de milliers de composés.

Pour la restitution structurale, c'est-à-dire pour la recherche de formules développées définies ou de familles de formules possédant en commun une ou plusieurs sous-structures données, le système DARC dispose d'un logiciel très élaboré, qui met en jeu un ensemble d'écrans structuraux originaux et délivre, sans bruit ni silence, les formules recherchées. Ces dernières peuvent donner accès à des références bibliographiques ou à des informations

associées ; elles peuvent aussi être éditées en 2D (stéréochimie en option).

Le langage d'interrogation est disponible dans des versions alphanumériques et/ou graphiques, pour des recherches par trains de questions (batch) ou sous forme de dialogue interactif (recherche conversationnelle). Toutes ces versions sont portables et n'exigent aucune connaissance du code DARC, entièrement transparent dans les échanges homme-machine. A l'occasion du projet PISCINE, un effort particulier a été fait pour les programmes d'édition graphique, qui produisent à partir des codes enregistrés des représentations structurales très conformes aux habitudes des chimistes.

II - SYSTEME SYNTAX

Ce système, qui a été conçu pour une gestion dynamique des banques de données, n'impose pas d'établir a priori une structure rigide du contenu des articles, ni de prévoir une organisation définitive des données, ce qui est particulièrement utile pour des banques dont la définition et le stock informationnel, comme ceux d'ECDIN, sont appelés à une évolution rapide au cours du temps.

Pour PISCINE, le système, à l'état initial de prototype, a été entièrement réécrit et testé, sur CII 1070 puis sur CII IRIS 80, par application sur un lot de données variées rassemblées pour ECDIN. Son langage d'interrogation, proche du langage naturel et, par la création de "synonymes", très voisin de n'importe quelle langue usuelle, permet un accès conversationnel très rapide aux données stockées. Une interface SYNTAX/COBOL autorise l'application de nombreux types de traitement aux données extraites par SYNTAX.

Contractant : Université Louis Pasteur - 67000 STRASBOURG (France)

N° du Contrat : 118-75-1 Env.F.

Chef du Projet : Professeur A. DELUZARCHE

Titre du Projet : CONTRIBUTION A L'ETABLISSEMENT D'UNE BANQUE DE
DONNEES SUR LES PESTICIDES ET CERTAINS MICRO-
POLLUANTS.

L'étude visait à fournir des données pour tester l'enregistrement sur ordinateur dans le cadre du Projet pilot ECDIN. Elle a couvert cinq directions.

I. CARACTERISTIQUES DE PESTICIDES

Pour chacun des 95 composés cités à l'annexe I a été établie une fiche précisant :

1. Identification

- a) Numéro aux Chemical Abstracts
- b) Noms communs et commerciaux en France
- c) S'ils sont disponibles, les numéros d'identification chimique de la C.E.E. et du Conseil de l'Europe.

2. Propriétés physiques et chimiques (dans les domaines suivants)

- a) Formule moléculaire
- b) Poids moléculaire
- c) Point d'ébullition
- d) Point de fusion
- e) Pression de vapeur
- f) Température de décomposition
- g) Solubilité dans l'eau
- h) Solubilité dans les solvants organiques.

3. Méthodes analytiques générales

II. DISPERSION ET TRANSFORMATION DANS L'ENVIRONNEMENT (sur 20 composés, annexe II)

Rapports schématiques comportant références bibliographiques et éventuellement en tableaux les dégradations dans l'environnement.

Ils signalent

- a) Synthèse dans l'environnement
- b) Les voies de dispersion
- c) La persistance

- d) La dégradation abiotique
- e) L'absorption biologique, le métabolisme et l'excrétion
- f) La rétention biologique et l'accumulation
- g) Les taux dans l'environnement
- h) L'absorption par l'homme et les animaux domestiques.

III. METHODES ANALYTIQUES

Deux rapports détaillés ont concerné les méthodes de dosage

- a) Du diméthylmercure et de l'ion méthylmercure
- b) Des alkylplomb.

IV. DISPERSION DANS L'ENVIRONNEMENT

Elle peut s'étudier

- a) Au niveau producteur (production, importation, exportation)
- b) Au niveau utilisateur.

La première méthode s'est révélée difficile devant la réticence assez générale des producteurs français à fournir des informations. Les données obtenues (année 1973) concernent des productions groupées et non détaillées en général au niveau des produits ou de Sociétés.

L'étude des statistiques douanières françaises (Statistiques du Commerce Extérieur de la France, année 1973) est relativement décevante. Regroupements trop vastes (par exemple : Dérivés halogénés des hydrocarbures cyclaniques et cycloterpéniques, autres. Herbicides) où sont confondus des produits de toxicité ou rémanence très variable. Quelques rares composés sont détaillés (par exemple : DDT, Diazinon, Simazine).

La France a été située du point de vue mondiale (1975) et européen (24%).

Pour le deuxième mode d'étude, nous avons présenté un modèle d'examen extrapolable à d'autres régions et concernant l'Alsace (France) (départements du Bas-Rhin et Haut-Rhin) territoire en bordure du Rhin.

Nous avons mis en évidence l'existence de trois circuits différents de distribution des produits phytosanitaires.

En tenant compte des pourcentages en matières actives des produits phytosanitaires et en connaissant le pouvoir de pénétration du marché, nous avons pu estimer la nature et quantités des produits utilisés en Alsace. L'étude a été complétée par les données fournies par une exploitation type de 50 hectares.

Cette deuxième approche bien qu'elle n'ait concerné qu'une région française nous paraît supérieure à la première.

- a) Elle permet d'établir la pollution par bassin hydrographique ;
- b) Elle permet d'envisager dans certains cas de comparer pour un même composé (par exemple : méthylmercure) les pollutions d'origine industrielle, domestique, agricole.
- c) Complétée par des analyses convenablement choisies (terrains tests, eaux, vases, êtres vivants (lait de vache et de femmes pour les organochlorés), elle permettrait de suivre la pollution dans les chaînes alimentaires, d'en préciser les causes et de faire localement les recommandations

nécessaires au niveau de l'emploi de certains composés phytosanitaires.

Dans cet esprit le rapport avant l'accident de Séveso attirait l'attention sur l'utilisation du 2,4,5 T et des dosages de la dioxine.

V. LES PESTICIDES EN FRANCE DU POINT DE VUE DE LA REGLEMENTATION ET DE LA TOXICOLOGIE. Trois rapports concernent :

- a) La réglementation en France sur les pesticides (jusqu'en 1974)
- b) La liste des Centres antipoisons
- c) Une enquête sur les accidents dûs en France aux pesticides (1970 - 1975).

Annexe I

LISTE DE PRODUITS AYANT FAIT L'OBJET D'UNE FICHE

Atrazine	Méthoxychlor	Phorate
Chlorazine	Toxaphene	Phosalone
Propazine	Azinphos-éthyl	Phosphamidon
Simazine	Azinphos-méthyl	Phoxim
Chloroxuron	Bayrusil	S.D. - 8447
Diuron	Betasan	Supracide
Linuron	Bromophos	Sytam
Monolinuron	Butonate	Thiocron
Monuron	Carbophenothion	Thiometon
Neburon	Cyolane	Vamidothion
2,4 - D.	Demeton	Wepsyn
2,4 - D.E.P.	Demeton - O	
Dichlobenil	Demeton - S	
M.C.P.A.	Demeton-S-Methylsulfone	
2,4,5 - T.	Dialifor	
Barban	Diazinon	
Bi. P.C.	Dicaptan	
Sulfallate	Dichlofenthion	
Diallate	Dichlorvos	
Agritox	Diméthoate	
1,2-Dibromo-3 Chloro-propane	Dipterex	
Chlorophacinone	Disulfoton	
D.C.P.A.	Dithione	
Kepone	Dowco - 199	
Kelthane	Endothion	
H.C.B.	Fenthion	
P.C.N.B. (Quintozene)	Folimat	
Sinbar	Folithion	
D.D.T.	G.C. 4072	
D.D.E.	Gophacide	
D.D.D.	Hinosan	
Chlordane	Jodofenphos	
Endosulfan	Malaixon	
Endrin	Malathion	
H.C.H. - α	Menazon	
H.C.H. - β	Meta - Systox	
H.C.H. - γ	Mevinphos	
Heptachlor	Oxydemeton - Méthyl	
Heptachlor Epoxyde	Oxydisulfoton	
Isodrin	Parathion	
Aldrin	Parathion - Méthyl	
Dieldrin	Phenkapton	

Annexe II

Dieldrine	Aldrine
Atrazine	Diazinon
Méthoxychlor	Heptachlor
Alkylmercures	Heptachlor Epoxyde
HCH (β γ)	Malathion
Méthyl Parathion	Azinphos-Ethyl
Ethyl Parathion	Azinphos-Methyl
2,4 D	Malaixon
Endosulfan	Dichlorvos

Contractor : MONTEDISON - Largo Donegani 1/2
20121 MILANO - Italy

Contract : n° 124-75-1 ENV I

Project Leader : Dr. Tiziano Garlanda

Title of project : Contribution to ECDIN

For the 87 chemicals listed below, data sheets containing information on chemical, physical and biological properties, analytical procedures, produced quantities and manufacturing processes were drawn up.

DIFLUORO DICHLOROMETHANE	METHACRYLIC ACID
METHYLENE CHLORIDE	PHTHALIC ANHYDRIDE
1,2-DICHLOROETHANE	CETYL ALCOHOL
1,1-DICHLOROETHANE	FAC
PENTACHLORO ETHANE	ROGOR
HEXACHLORO ETHANE	PHENTOATE
TRICHLORO METHANE	ZIRAM
CHLORODIFLUOROMETHANE	DODINE
VINYL BROMIDE	ZINEB
ACETYL CHLORIDE, CHLORO-	DIMETHYL SULFOXIDE
TRICHLOROFUOROMETHANE	DODECYLBENZENE
1,1,2-TRICHLORO-1,2,2-TRIFLUORO-ETHANE	PIPERAZINE
BENZYL CHLORIDE	2-METHYL-5-ETHYLPYRIDINE
ETHYLENEDIAMINE	FURFURYL ALCOHOL
HEXAMETHYLENE TETRAMINE	CYCLOHEXANONE
P-PHENYLENEDIAMINE	METHYL CELLUSOLVE
TRITHANOLAMINE	BUTYL CELLUSOLVE
DIETHYLENE TRIAMINE	ETHANOL, 2-ETHOXY
DI-ISOPROPYLAMINE	ACETIC ACID BUTYL ESTER
TRIETHYLAMINE	DIETHYL ETHER
OXALIC ACID	TRIETHYLENE GLYCOL
PENTACHLOROPHENOL, SODIUM SALT	ACETONITRILE
ETHYL ACRYLATE	DIMETHYLFORMAMIDE
ISOBUTYL ACETATE	ETHYL ACETATE
METHYL ACRYLATE	METHYL ACETATE
STEARIC ACID	DIMETHYL ETHER
MALEIC ACID, DIBUTYL ESTER	O-XYLENE
SODIUM ACETATE	P-XYLENE
TANNIC ACID	DIETHYLENE GLYCOL, MONOBUTYL ETHER
TARTARIC ACID	DIPROPYLENE GLYCOL

TRIPROPYLENE GLYCOL
SODIUM NITRITE
SODIUM NITRATE
SODIUM FLUORIDE
SODIUM BISULFITE
NITROUS OXIDE
BORIC ACID
SULPHUR
PHOSPHORUS
HYDROGEN CYANIDE
HYDROQUINONE
DODECENE (TETRAPROPYLENE)
ACETONE CYANHYDRIN
ACETOPHENONE

BENZAL CHLORIDE
PROPIIONALDEHYDE
BENZOYL PEROXIDE
BUTANE
STYRENE, ALPHA-METHYL-
PROPANE
CINNAMIC ALDEHYDE
NICOTINIC ACID
NICOTINAMIDE
NICOTINIC ACID METHYL ESTER
PALMITOYL CHLORIDE
CHLORAMPHENICOL

Contractor : National Institute for Water Supply, P.O. Box 150
2260 AD Leidschendam, The Netherlands

Contract n^o : 117-75-1 ENV N

Project leader : Ir. A.H. Nettenbreijer

Title of project: Databank for threshold odour concentrations
of chemicals in water and air

Within the framework of this project, a computerized file of threshold odour concentrations of chemicals on water and air and other relevant information was established, to be incorporated in the pilot project of a data bank on environmental chemicals (ECDIN) of the European Communities.

It provides early information about chemicals in public water supply systems affecting the taste and odour of the water and it expedites the solution of difficult surface water pollution problems.

Information on nearly 500 compounds has been collected from literature and from laboratory tests with an odour panel of the National Institute for Water Supply in The Netherlands.

Besides the lowest concentration of a substance in water which can be smelt by 50% of an "odour panel" (this is the so-called "detection" threshold) a word is introduced to describe the odour of each compound. These "qualifiers" are:

- 1: rotting, sulphidic
- 2: oily
- 3: disinfectant
- 4: muddy, earthy
- 5: floral, fruity, fragrant
- 6: metallic
- 7: green, woody
- 8: pungent
- 9: fishy, amine

These qualifiers facilitate the identification of compounds. Several other physical and chemical characteristics are given as well. The complete list of characteristics is as follows:

- ECDIN number
- preferred systematic name
- other names (e.g. trade-names)
- chemical abstracts service registry number
- wiswesser line notation
- molecular formula
- molecular weight
- melting point
- boiling point
- threshold odour type: detection/recognition
- modality: taste/odour
- medium: water/air
- purity: chemical pure /pure/gaschromatographically pure/
not specified
- odour description from literature
- odour description (numerical)
- threshold value
- reference in literature to threshold values

When the threshold values are divided into modality type (odour/taste) and threshold type (detection/recognition) the following survey can be made (see table 1).

Table 1: Survey of available types of sensory threshold values for chemicals in water and air.

Types of threshold data			
I odour threshold	detection	320	190
	recognition	19	177
II taste threshold	detection	85	-
	recognition	6	-

In case insufficient information on a compound was available, it was deleted from the list. Data were also deleted in case they were corrected after the measurements. In total 483 compounds have been processed.

October 1977.

Publication

A.H. Nettenbreijer, G.J. Piet and B.C.J. Zoeteman.
Databank for threshold odour concentrations of chemicals in water and air.

R.I.D. Quarterly report n° 9, March 1977

Contractor: UNITED KINGDOM ATOMIC ENERGY AUTHORITY
HARWELL LABORATORY

Contract N°: 067-74-1 ENV UK

Project Leader: F.S. FEATES

Title of project: Contribution to the ECDIN + pilot project
Summary Report of work carried out by Harwell
during the two year contractual period
ending 31.12.75

Introduction

In accordance with the agreed research programme Harwell accepted responsibility for collating data appropriate to Schedules 1, 3, 6 and 7 of the ECDIN study list of attributes (dated 8.5.74). These covered identification, physical and chemical properties, transport, packing, storage, handling and hazards, and use and disposal data.

This programme of work required an extension of the information already held in the Harwell Chemical Data Bank and the inclusion of additional compounds selected by the Commissions Services. The classes of compounds selected for the ECDIN pilot programme included carbamates, organo-phosphorous compounds and pesticides. It was anticipated that, subject to data availability, the four schedules would be drawn up for about 2000 compounds. Emphasis would be placed on covering as many compounds as possible rather than to attempt comprehensive searches for obscure information.

Data Collection

In order to test the proposed data forming procedure an initial study was carried out in collaboration with United Kingdom Chemical Information Services, who were also engaged on the ECDIN programme,

to collate data for 46 chemical compounds of the classes intended for the ECDIN pilot programme. These compounds were selected on the basis that most of the attributes required were known to be readily available at Harwell.

The results of this study enabled various improvements to be recommended to the ECDIN list of attributes especially in data coding.

Since comprehensive data for many of the selected compounds proved to be non-existent, emphasis was placed on considering as many compounds as possible rather than attempting to complete all attributes for fewer substances. It was found to be increasingly difficult to obtain information on approximately half of the 1,000 compounds initially selected. This was thought to be due to many reasons including products on the list being superseded, some compounds listed not existing commercially, and others having general names for example:- polychlorobiphenyls with confusing CAS registry numbers. In view of these difficulties approval was sought and obtained to provide data on substances held in the Harwell Data Bank. This action enabled a wide selection of chemical products to be covered.

By September 1975 Category 1, 3 and 6 data for about 650 compounds had been collected. Further searching was then discontinued to allow for conversion of data collected, into machine form, correction of character errors and assimilation with data for the Harwell Data Bank. In addition to data punching it was necessary to write additional computer programmes in order to convert much of the data already coded in the original format and field descriptions, to the more recent ECDIN input format (a new requirement).

It was agreed with the Commissions Services that Harwell would concentrate mainly on the waste disposal aspects of category 7 and to advise on a suitable format. Examples to waste disposal data for some compounds was subsequently submitted for evaluation and finalising an acceptable format.

Harwell has used this to provide waste disposal data for a few compounds. Owing to financial limitations it was not possible to provide further data in category 7 during this contract.

Project discussion and advice

Throughout the programme discussions were held with UKCIS and representatives of the Commission to ensure satisfactory progress. The difficulties encountered during the continuous evolvement of ECDIN required Harwell to comment and advise on many aspects of the data collection, particularly with the detail of Categories 3 and 6. Necessary changes in formatting requirements have resulted in fewer compounds being covered than was planned. However, the total number of compounds for which data has been supplied exceeded 2000, the balance being made up from the Harwell Data Bank. Data was supplied on separate magnetic tapes for ECDIN data (ECDIN input format) and Harwell data (Harwell output format). In addition samples of the Harwell data together with format descriptions have been sent to UKCIS to assist with the development of the 'exchange format'.

Contractor : The Chemical Society

Contract n^o 092-74-5 ENV UK

Project Leader : D.C. Veal & J.E. Blackmore

Title of Project : Data collection, and transmission using an exchange format.

1. The objectives of the work can be defined as follows:

(a) To design a computer file format suitable for the exchange of data on chemical compounds in machine-readable form. The format should accommodate all ECDIN data elements and should be flexible enough to handle other data that may be required in the future or that may be held by other sources contributing to the network.

(b) To test the format by converting data in the ECDIN format to the exchange format, by converting other files into the exchange format and by converting data from the exchange format into the ECDIN format.

(c) To collect input data relating to compound identification, chemical structure, physical and chemical properties and chemical analysis, data and methods by generating Registry Numbers for the ECDIN compounds, supplying systematic nomenclature in machine-readable form through the exchange format, supplying data in the above areas from the Registry System, and generating substructure screens for the compounds.

(d) To supply name-match and identification data for additional compounds as requested by ECDIN.

(e) To make a preliminary study of the problems of the standardisation of data content.

Full details of these objectives may be found in Refs. 1 and 2.

2. Lists of chemical and trade names supplied by both ECDIN and the Atomic Energy Research Establishment, Harwell were name-matched using the CAS Registry System (3) to identify their Registry Numbers. Those compounds that failed to match were submitted to structure match. WLN's for several hundred compounds (requested by Ispra) were coded and despatched. Following the completion of the name-match and structure-match, a complete ECDIN Number - Registry Number Concordance was produced. A set of substructure screens for the ECDIN file were produced, and trial searches were made to identify bibliographic material on selected compounds. A comprehensive report was produced (4) which detailed the exchange format specifications and programs to convert CAS Registry data and ECDIN data into

the format were written and tested. Further programs were written to allow conversion of Harwell data and to convert from the exchange format into the ECDIN format.

Using the exchange format, all the required Registry data on 3516 compounds was supplied to the Commission's services.

3. All the objectives defined in Section 1 have been met with the exception of 1(e). Some preliminary work has been carried out, but no firm conclusion has yet been reached. Work on this is continuing.

A paper detailing the work done on the exchange format concept has been prepared for publication (5).

References.

- (1) Contract between the European Economic Community and the Chemical Society O92-74-5 ENV UK.
- (2) Supplementary agreement No. 2 to contract O92-74-5 ENV UK between the European Economic Community and the Chemical Society.
- (3) Dittmar, P.G., Stobaugh, R.E., Watson, E.C. The Chemical Abstracts Service Chemical Registry System, I. General Design.
- (4) Specification of ECDIN Exchange File Format. UKCIS 1975 Ref El.750611.
- (5) Proctor, D.J., Robson, A., Veal, M.A., Petrie, J.H., Town, W.G. Development of an Exchange Format for the European Environmental Chemical Data and Information Network (ECDIN). To be published in Information Processing and Management.

Contractor : SRI International, Croydon, England

Contract n° : 112-75-1 ENV UK

Project Leader : V. von Schuller-Götzburg

Title of project : Elaboration of a format and supply of data on production and use of chemicals (contribution to ECDIN).

Brief general description of work carried out and results :

The work carried out by SRI International under the above-mentioned contract essentially consisted of two major tasks :

1. The preparation of data related to the commercial production and use of hundred important chemicals as a pilot input to the data bank of the Environmental Chemicals Data and Information Network (ECDIN).
2. The establishment of a workable format for the incorporation of the data directly into the computerized data bank ECDIN, in cooperation with the Commission's services.

The first step of the first task was the selection of the chemicals to be studied in this research program. This selection was based on a list of important environmental chemicals that had been submitted for discussion by the Commission's services and on a number of similar lists that had been prepared by SRI in the course of earlier chemical environmental projects. The most interesting chemicals from the environmental point of view that were on these SRI lists were grouped into a number of additional lists to which other compounds, that had been identified by screening the available information, were added. From these lists hundred chemicals to be studied were selected, based on criteria such as known or assumed toxicity, order of magnitude of quantities produced and consumed, main uses, a special interest in certain classes of compounds by the authorities involved and the relative ease or difficulty in obtaining or estimating the data desired for a candidate chemical.

For the hundred chemicals selected, the following data were collected by SRI, as far as they could be obtained from SRI's own files or from the manufacturing companies under the time and money constraints established by the funding of the project : manufacturing process ; producers ; production ; foreign trade ; domestic supply ; transport, packing, handling, and storage ; and use and disposal. These classes of information conform generally to a modified version of the document "List of Attributes for ECDIN" of 8.5.1974. For three of the selected chemicals, all these data were developed completely by SRI, in order to provide ECDIN with three examples of a complete data file.

For many of the chemicals selected for study only very few data were available. SRI developed qualified estimates for these chemicals wherever this was possible with a reasonable effort.

Parallel with the selection of the hundred chemicals to be studied by SRI, a workable format for the direct incorporation of the data to be developed by the research project into the computerized data bank of ECDIN was established by SRI in close cooperation with the services of the Commission. This format was based on the data categories agreed upon and it was developed by using the experience SRI had acquired when working on similar tasks for other chemical environmental projects. As part of this format, a system of computer codes was established for the different data categories and for the fields of the data bank corresponding to the data collected or developed by SRI. Furthermore, a set of special forms was prepared using the computer codes that had been established, in order to facilitate entering the data into the computer in machine readable form. The data that had been developed by SRI on each of the hundred chemicals were supplied in the form of individual dossiers made up of these special forms.

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