EUROPEAN COMMISSION

Reports of the Scientific Committee for Pesticides

(Fourth series)

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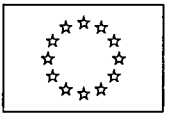
Report



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FOREWORD

The Scientific Committee for Pesticides was set up by Commission Decision 78/436/EEC of 21 April 1978 (OJ N° L 124 of 12.05.78, p. 16) in order to provide the Commission with informed opinions on scientific and technical matters relating to the use and marketing of pesticides and to their residues, particularly in food and feedingstuffs.

The members of the Committee are independent and highly qualified in the fields of applied biology, toxicology, ecotoxicology and chemistry. The Secretariat of the Committee is provided by the Commission's Directorate-General for Agriculture.

The Committee's third series of reports, published in this volume, relate to questions put to it by the Commission on the safety in use, for man and environment, of certain pesticides and on the maximum permitted levels of their residues in foodstuffs. Questions in this connection had arisen in the course of the Commission's work on the approximation of Member States' legislation concerning pesticides.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF CHLOROTHALONIL

(Opinion expressed by the SCP on 11 February 1992)

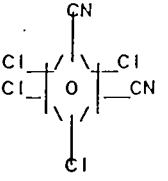
BACKGROUND AND TERMS OF REFERENCE.

Chlorothalonil is authorized for use as a fungicide in all Member States on a wide range of fruit and vegetable crops. It has been evaluated by the Joint Meeting on Pesticide Residues (JMPR) in 1974, 1977, 1979, 1981, 1983, 1985, 1987 and 1990 (1). The temporary acceptable daily intake (ADI) of 0.03 mg/kg bw estimated in 1974 was reduced in 1981 and 1985 to 0.005 mg/kg bw and 0.0005 mg/kg/bw respectively due to inadequate metabolism data and concern for oncogenicity. In 1987, additional data allowed the temporary ADI to be increased to 0.003 mg/kg/bw. Since then additional data has become available which permitted the 1990 JMPR to estimate an increased ADI of 0.03 mg/kg/bw.

In the context of its work relating to the establishment of Community maximum pesticide residue levels in various foodstuffs covered by Community legislation, the Commission requested the Scientific Committee for Pesticides to review the basis of the ADI, paying particular attention to the carcinogenic effects observed in laboratory animals and the relevance of these effects for man.

1. TOXICOLOGICAL ASPECTS.

1.1. PHYSICAL-CHEMICAL PROPERTIES



Chlorothalonil is the common name for 2,4,5,6-tetrachloro-isophtalonitrile ($C_8Cl_4N_2$, CAS n°: 1897-45-6).
The molecular weight is 265.92.
Its colourless crystals (melting point 250°C) are slightly soluble in water (0.6 mg/l at 25°C).
It is soluble in organic solvents, and its log Pow is 4.4.
It is easily adsorbed on suspended particulates. The partition coefficient (log Kac) is 5.7.
It is stable to UV light and in alkaline aqueous medium.

1.2. METABOLIC STUDIES

1.2.1. Oral route

Rats

In vitro experiments on the incubation of ^{14}C-chlorothalonil with stomach and intestinal mucosal cells show that ^{14}C-chlorothalonil is not absorbed as such by the gastro-intestinal tract and must be converted to polar metabolites prior to its absorption (2). Studies, in which radio-labelled chlorothalonil introduced into "sacs" formed from the upper section of the small intestine of the rat show that only the polar metabolites pass through the intestinal mucosa and are transferred to the serosal side of the sac. No chlorothalonil passes through the mucosa (3).

Radio-labelled chlorothalonil administered orally to rats at doses of 5, 50 and 200 mg/kg/bw is only partially but rapidly absorbed by the gastro-intestinal tract . The concentration in blood (within 30 minutes) is proportional to the dose but non linear between 50 and 200 mg/kg/bw dosages showing some saturation of the metabolic capacity to form polar metabolites. The percentage of urinary elimination of the compound goes up to 6 % of the administered dose but appears to decrease with increasing dose levels. Most of the radioactivity is eliminated in the faeces (4).

The absorbed radio-material is distributed among body tissues. The kidney contains the highest concentration of radioactivity (up to 7 % of the dose), the radio-labelled compound being bound to kidney proteins. At a subcellular level, approximately 81 % of the radio-label present in the kidney is found in the cytosol . More than 10 % of the compound found in the organelles of the kidney cells is associated with mitochondria (5).

The identification of urinary and biliary metabolites shows that the major pathway in the rat metabolism involves the formation of conjugates with glutathione (6). Degradation of the conjugate derivatives gives rise to generation of thiol metabolites including the 4,6-dithiol derivative of chlorothalonil which is known to inhibit mitochondrial oxygen consumption and prevent energy production through ATP (7).

Rats germ free

A single dose of ^{14}C-chlorothalonil at 50 mg/kg/bw was given orally to germ-free Sprague-Dawley rats. Urine and faeces were collected at 24 and 96 hours and blood, kidneys and the carcass at 98 hours. Urinary excretion of radioactivity accounted for about 3 % of the administered dose and the fecal excretion for 84 % . Blood levels accounted for 0.01 % , the kidneys containing 0.05 % . Faecal and urinary excretion of the test material occurred mainly in the first 24 hours after dosing. Urine samples were analyzed for thiol derivatives. Mono-thiol was not detected in any sample. Dithiol was detected only in one 24-48 h sample and the trithiol in two samples of 0-24 h and two of the 24-48 h period (3 positive rats out of 9). The highest concentration of thiols detected accounted for 0.053 % of the administered dose (8).

In a comparative study with non germ free rats it was found that 1.7 % of the dose was excreted as thiols. Thiol excretion was much less in germ free rats than normal rats.

Dogs

Two male beagle dogs were given ^{14}C-chlorothalonil by gelatin capsule at 50 mg/kg bw. Samples were collected every 24 hours for a period of 10 days for urine and for 12 days for faeces. Recovery of test material was virtually complete. Most of the material was excreted in the faeces with only small amounts in urine (0.2 and 0.3 %). Urine samples were analyzed for thiol derivatives and none were detected at the detection limit of 3 ng/ml (9).

A second study was performed on 3 dogs given a single oral dose of the same radio-labelled material at 50 mg/kg bw. The animals were observed for a period of 8 days, urine being obtained by catheterization. Faecal radioactivity in the first 24 hours accounted for 60 to 96 % of the nominal dose administered and a further amount during days 2 and 3. Urinary excretion accounted for 0.6 to 1.7 % of the nominal dose which was recovered predominantly in the first 24 hours, and mostly during the first 10 h after dosing. Urine was analysed for thiol derivatives and none were detected in any of the samples (10).

Monkeys

Four male Chinese Rhesus monkeys were given the same radio-labelled material by gavage at 50 mg/kg bw. Urine and blood were collected by catheters for 48 h after dosing and the animal housed in metabolism cages up to 96 h after dosing.

Blood concentrations versus time plots, as well as blood elimination half-life (from 6.9 to 35 h), showed great variability between animals, . Over 96 h, 1.75 - 4.13 % and 52-91 % of the administered doses were excreted in urine and faeces respectively. Monothiols were not detected in any sample, dithiols were characterized in the urine of one monkey and trithiols in all urine samples but the total amount was very low, 0.001 to 0.01 % of the administered dose (11).

1.2.2. Dermal Route

Rats

A total of twenty male CD Sprague-Dawley rats were treated dermally with ^{14}C-chlorothalonil in acetone at a dose of about 5 mg/kg bw. Urine was collected from 4 metabolic cages each containing 5 animals. Considerable variability was noted in results, urinary excretion in the first 48 h accounted for about 3 % of the administered dose with total thiols representing 0.001 to 0.07 %. In the 0-24h period samples, monothiols were detected in samples from one cage, dithiol samples from two cages and trithiols in samples from all cages (12).

Monkeys

Four monkeys were treated dermally with 5 mg/kg bw of ^{14}C-chlorothalonil under a non-occlusive patch. After 48 hours the patch was taken off and the skin was washed. About 90% of the dose was recovered from the surface and about 2.26% was completely absorbed through the skin. The urine contained 1% of the dose, but methylated mono,di- and trithiols were not detectable in the urine. This means that significantly less than 0.008% of the dose was excreted as thiols (13).

1.2.3. In vitro metabolism.

Liver and kidney mitochondrial preparations (Sprague-Dawley rats) were incubated with sulfur derivatives of chlorothalonil such as the monothiol, the dithiol, the monoglutathione and the diglutathione analogs. Based on oxygen consumption, none of the glutathione analogs had an effect on oxygen uptake whereas
the dithiol inhibited respiration in liver and kidney and the monothiol in liver only. The authors suggest that these results support the fact that glutathione conjugates, while not toxic per se, may be metabolized in the kidneys to nephrotoxic thiols (7).

1.3. TOXICITY.

The acute oral LD50 of chlorothalonil for rats has been estimated above 10,000 mg/kg. The LC50 by inhalation in rats (4 hours) is 0.9 mg/l. The percutaneous LD50 is above 10,000 mg/kg (rabbits).
Chlorothalonil is irritating to the eye of rabbits (14), and to skin after repeated applications for 21 days (15).

Earlier chronic toxicity studies have been reviewed by the 1974 JMPR. Since then newer carcinogenicity studies have become available.

1.4. TUMORIGENICITY

Several studies in rats and mice have been completed to investigate the tumorigenic potential of chlorothalonil.

RATS

1.4.1. National Cancer Institute (1978).

In an inadequate carcinogenicity study (18), chlorothalonil was administered to male and female Osborne Mendel rats in the diet at dose levels of 5063 and 10126 ppm (approximately 250 and 500 mg/kg bw/day).
Chlorothalonil administration was associated with tumours in the tubular epithelium of the kidneys at each dose.

1.4.2. Fermenta ASC Corporation (1985).

Chlorothalonil was administered (19) to Fischer 344 rats in the diet at dose levels of 40, 80 and 175 mg/kg bw/day for 27 months in males and 30 months in the females.

Kidneys: a statistically significantly higher incidence of tubular adenomas and carcinomas in the kidneys was observed in rats of all treated groups when compared to the control group, with the exception

of the 40 mg/kg bw/day females group.

In addition to these renal tumors, proximal tubular hyperplasia was present in the kidneys of male and female rats from all dose groups. The severity of the hyperplasia increased in a dose-related fashion. A correlation between renal tumors and this hyperplastic lesion was evident, confirming the conclusion that the tubular hyperplasia was preneoplastic.

Forestomach: an increased incidence of hyperplasia and hyperkeratosis of the squamous mucosa was observed in all treated groups compared with the control group and the severity of the lesion increased in a dose-related fashion. Increased incidences of focal necrosis or ulceration of the squamous mucosa and submucosal inflammation also were observed. These effects in the forestomach were considered to be related to chronic irritation of the mucosa caused by the test material.

In addition, papillomas and carcinomas were present in the forestomach of treated rats. The incidence was low and not statistically significant in the low and the mid-dose groups but significant in the high dose females group.
The forestomach tumors are considered to be sequelae to the hyperplasia/hyperkeratosis noted in the squamous mucosa and therefore related to the irritant properties of the test material.

1.4.3. Fermenta ASC Corporation (1989).

Another study in Fischer 344 rats (20) was conducted to determine the no-effect-levels (NOAEL) on one hand for hyperplasia and tumors in the kidney and on the other hand for irritation and tumours in the forestomach. Chlorothalonil was given to males up to 26 months and to females for 29 months at dietary dose levels of 1.8, 3.8, 15 and 175 mg/kg/day .

Histopathological evaluation of the kidneys showed that focal epithelial hyperplasia was significantly increased in males and females at 175 mg/kg bw and slightly increased in females at 3.8 and 15 mg/kg bw. Kidney tubular carcinomas were observed in both sexes at 175 mg/kg bw but in no other group. Tubular adenomas were seen in 1/55, 1/54, 1/54, 3/54 and 17/55 in males at 0, 1.8, 3.8, 15 and 175 mg/kg bw respectively. In females, 24/55 were found at 175 mg/kg/bw only.

Epithelial hyperplasia and hyperkeratosis of the non-glandular stomach were seen at doses of 3.8 mg/kg bw and higher and were dose related in incidence and severity. Non-glandular papillomas in the stomach were seen in 0, 0.3, 2 and 5 males and 1, 1.2, 4 and 7 females at 0, 1.8, 3.8, 15 and 175 mg/kg bw respectively. No squamous cell carcinomas were observed in males, but one was seen in each of the control and 15 mg/kg bw female groups and 3 in the 175 mg/kg bw females.

In conclusion kidney and stomach tumours were observed at 175 mg/kg bw/day. There was a possible treatment related slight increase in kidney tubular adenomas and non-glandular stomach papillomas at 15 mg/kg bw/day, but no evidence of tumourgenicity at lower dose levels. The NOEL for hyperplastic lesions in kidneys and stomach was 1.8 mg/kg bw/day.

MICE

1.4.4. National Cancer Institute (1978).

In an inadequate carcinogenicity study (18), chlorothalonil was administered to B6C3F1 mice at dose levels of 2688 and 5375 ppm (approximately 385 and 770 mg/kg bw/day) in males and at dose level of 3000 and 6000 ppm (approximately 430 and 860 mg/kg bw/day) in females.

Chlorothalonil administration was not associated with renal tumour formation in mice in this study.

1.4.5. Diamond Shamrock Corporation (1983).

Chlorothalonil was administered in the diet (21) to Charles River CD-1 mice at dose levels of 750, 1500 and 3000 ppm (approximately equivalent to 125, 250 and 550 mg/kg bw/day) for 24 months.

Kidneys: an increased incidence of renal adenomas and carcinomas related to chlorothalonil administration was observed only in male mice. The incidence was low (10 % or less) , not dose-related and statistically significant only at the low dose level. Renal neoplasia was not observed in female mice.

Examination of the kidneys also revealed a distinct proximal tubular hyperplasia in males and females from all groups treated with chlorothalonil, incidence and severity being greater in males than in females.

Forestomach: The incidence of squamous cell tumors in the forestomach in male and female mice in the chlorothalonil-treated groups were higher than in the control group but statistically significant only in females of the mid-dose group.
Hyperplasia/hyperkeratosis of the mucosa of the forestomach were also noted in mice of both sexes in all treated groups.

1.4.6. Fermenta ASC Corporation (1987).

Another 2-year study in male Charles River CD-1 mice (22) was conducted in order to establish NOEL's. The dietary dose levels of chlorothalonil were 10, 40, 175 and 750 ppm. At week 18 , the low dose level was increased to 15 ppm to assure that the substance consumption in this group would be at least 1.5 mg/kg bw/day throughout the study.

Kidneys: No treatment-related renal tumours were observed in mice from any of the groups, including the high dose group. In the 1983 tumorigenicity study in mice, treatment-related effects did occur in the kidneys of males at 750 ppm. Therefore, the NOEL for renal tumors in mice must be fixed at 21.3 mg/kg bw/day (175 ppm) in males and 550 mg/kg bw/day (3000 ppm) in females.

A clear increased incidence and severity of tubular hyperplasia was revealed in the 750 ppm dose group and a slight one was noted in the 175 ppm dose group. Based on these results the NOEL for tubular hyperplasia is 40 ppm equal to 4.5 mg/kg/day.

Forestomach: The NOEL for tumours in the forestomach was evaluated at 21.3 mg/kg bw/day . Histopathologic evaluation of the forestomachs

demonstrated that the NOEL for hyperplasia/ hyperkeratosis was 10ppm equal to 1.6 mg/kg bw/day.
Since this study was only on male mice, it must be assumed that females and males are of equal sensitivity.

1.5. INVESTIGATIVE STUDIES.

The pathologic effects in the kidney associated with chlorothalonil administration have been further evaluated in three sub-chronic toxicity studies in rats.

1.5.1. Charles River CD rats (1985).

Charles River rats (23) received dietary dose levels of chlorothalonil equivalent to 1.5, 3.0, 10 and 40 mg/kg bw during a 13-week period.

A high incidence of hyperplasia of the epithelium of the proximal tubules was observed only in the males of the high dose group.
Examination of the kidney of the rats receiving the dose of 40 mg/kg bw/day was made by electron microscopy. The only alteration noted was the presence of irregular, intracytoplasmic inclusion bodies in otherwise normal cells in the proximal tubules of male rats. The phenomenon was not observed in females. The toxicological significance of the inclusions is unclear.

1.5.2. Fischer 344 rats (first experiment-1987).

Chlorothalonil was administered in the diet to rats at dose levels equivalent to 175 mg/kg for 91 days (24).

In the kidney, vacuolar degeneration and loss of some proximal tubular cells were observed. Continued administration resulted primarily in tubular hyperplasia which may have been a consequence of the initial damage.

1.5.3. Fisher 344 rats (second experiment-1987).

A 90 day study in male Fischer 344 rats was performed (25). Equimolar doses of chlorothalonil (75 mg/kg bw/day) and of its monoglutathione conjugate (150 mg/kg bw/day) were administered to the animals daily by oral gavage. Metabolites indicative of a common metabolic pathway for both substances were found in urine.

Similar histopathologic effects were observed for the two substances in the kidney including tubular hyperplasia, tubular dilatation, vacuolar degeneration and interstitial fibrosis.

Hyperplasia/hyperkeratosis, erosions and ulcerations of the forestomach mucosa were observed only in the chlorothalonil-treated group, not in the other one. Forestomach lesions seem to be associated with chlorothalonil itself, the local irritant effects of which are well known.

1.6. MUTAGENICITY

The genotoxicity of chlorothalonil has been investigated with many tests which have been reported in reference (26) and are summarized below.

1.6.1. Gene mutation tests

1.6.1.1. Ames test.

Mutation assays with chlorothalonil were conducted with 5 strains of Salmonella typhimurium with and without metabolic activation. Doses ranged from 0.33 to 6.6 µg/plate. The results were negative. To investigate the mutagenic potential of possible metabolites and manufacturing impurities of chlorothalonil, a series of Ames tests with S.typhimurium were conducted with selected compounds without or with activation by S-9 homogenates from kidney (the target organ). Four manufacturing impurities and 13 known or potential metabolites have been tested. The results were negative for all compounds.

1.6.1.2. In vitro mammalian systems.

Chlorothalonil was tested in the Chinese hamster V-79 cells at a dosage level of 0.3 µg/ml without activation. Difficulties encountered with the high spontaneous mutation rates of these cells necessitated use of a second cell line (BALB/3T3). Gene mutation in the mouse fibroblast BALB/3T3 cells was evaluated at a dosage level of 0.03 µg/ml without and 0.3 µg/ml with activation. The results were negative.

1.6.1.3. In vivo host-mediated assay.

Mice were dosed on five consecutive days with 6.5 mg/kg bw/day of chlorothalonil. Eight strains of S.typhimurium were used. The result was negative.

1.6.2. Cytogenic tests.

In a dominant lethal assay male mice were treated with 6.5 mg/kg bw/day for five consecutive days and then mated for 8 weeks. No dominant lethal effects were observed.

A series of micronucleus assays was conducted in male rats (0, 8, 40, 200, 1000 or 5000 mg/kg bw/day chlorothalonil oral dose), in male mice and male Chinese hamsters (0, 4, 20, 100, 500 or 2500 mg/kg bw/day oral dose). All animals were dosed twice with a 24-hour interval between doses. There was no increased incidence of micronuclei in polychromatic erythrocytes in the bone marrow.

A chromosome aberration test was conducted in rats. Male rats were dosed in 2 different ways. In the first one, animals were dosed orally twice with a 24-hour interval between doses of 0, 8, 40, 200, 1000 or 5000 mg/kg bw/day. In the second one, a single oral dose of 0, 500, 2500 or 5000 mg/kg was given. In both studies no increased incidence of chromosomal aberrations was noted in bone marrow cells.

A chromosome aberration test was conducted in male mice with the same protocol but the doses were 0, 4, 20, 100, 500 or 2500 mg/kg bw given twice with a 24-hour interval, and 0, 250, 1250 or 2500 mg/kg bw in a single oral dose. The results were negative.

In a chromosome aberration study Chinese hamsters were dosed with 0, 8, 40, 200, 1000 and 5000 mg/kg bw/day for 2 days. The results were inconclusive.

A new chromosome aberration study in Chinese hamster included both an acute and a subchronic dosing regimen. In the acute study, animals were given a single oral dose of 0, 500, 2500 or 5000 mg/kg. In the subchronic study, the animals were dosed for five days with 0, 50, 125 or 250 mg/kg/day. Data suggested a marginal (non dose-related) increase in chromosomal aberrations 48 h after a single dose of chlorothalonil and with repeated doses. Results were considered equivocal.

An in vitro chromosomal aberration assay was conducted in Chinese hamster ovary (CHO) cells exposed to chlorothalonil at 0.03, 0.08, 0.15 and 0.30 μg/ml without metabolic activation and at 0.6, 1.5, 3.0 and 6.0 μg/ml with metabolic activation. Chlorothalonil was considered positive without metabolic activation and negative with metabolic activation.

1.6.3. DNA repair.

A DNA damage test has been performed in 2 bacterial systems using the disk diffusion method.

Salmonella typhimurium cells were exposed to 2, 10 and 20 μg per disk of chlorothalonil. Significant killing of the repair deficient bacteria was noted, indicating DNA damage.

Bacillus subtilis was exposed to doses ranging from 2 to 200 μg per disk. No DNA damage was noted because there was no difference between repair deficient and repair proficient strains.

Isolation of DNA from kidneys of rats orally administered ^{14}C-chlorothalonil indicate that the radio-label is not covalently bound to DNA (27).

1.6.4. Cell transformation test.

A cell transformation assay with F1706 P95 and H4536 P+2 cells was performed with technical chlorothalonil. The cells were cultured in medium containing 0.01, 0.1 or 1 ng/ml chlorothalonil. The cells were injected into newborn Fischer rats. Chlorothalonil was not a transforming agent and the cells exposed to the highest dose did not grow in the newborn rats.

1.6.5. Conclusions.

Chlorothalonil was found positive in the S.typhimurium assay for DNA damage but negative with B.subtilis.. An in vivo DNA binding study was negative and the AMES test is also negative.

An in vitro chromosomal aberration study in CHO cells of Chinese hamster ovary was considered positive only without activation. However, in vivo chromosomal aberration assays, chlorothalonil was equivocal in Chinese hamster but clearly negative in rats and mice.

Chlorothalonil is considered not to present a genotoxic risk to man.

2. GENERAL CONCLUSIONS

2.1. Dietary administration of chlorothalonil to rats and mice results in tubular hyperplasia in the kidneys and hyperplasia/hyperkeratosis in the forestomach. These changes are sometimes accompanied by tubular adenomas and carcinomas in the kidneys and papillomas and carcinomas in the stomach.

2.2. The results of the battery of tests indicate that chlorothalonil is considered not to present a genotoxic risk.

2.3. Hyperplasia/hyperkeratosis in the forestomach is considered to be caused by local irritation of the mucosa by chlorothalonil itself.

2.4. The specific nephrotoxicity of chlorothalonil appears to be related to a metabolic pathway involving conjugation with glutathione.

2.5. Mono-, di- and tri-thiol metabolites of chlorothalonil have been found in significant amounts in urines of rat orally administered chlorothalonil and not, or at a very much lower extent, in urines of germ-free rats, monkeys and dogs. In these species, the thiol metabolites are minor end-products of the metabolism of chlorothalonil. This finding could be related to the differences in the gut microflora of these species.

2.6. In man, the microbial population in the upper gastrointestinal tract is much smaller than in the rat. Therefore, it is probable that, as in dogs and monkeys, thiol metabolites of chlorothalonil would be formed to a much lesser extent in man than in the rat, and that man would therefore be expected to be less susceptible to the nephrotoxic effects associated with chlorothalonil.

2.7. Chlorothalonil is evidently tumorigenic in rats and mice but this finding is not associated with mutagenicity and a plausible mechanism of action has been demonstrated for the tumorigenic effects in both the kidneys and the forestomach. An ADI may therefore be calculated based upon no effect levels for the mechanism of action.

2.8. The rat is the most susceptible species for tumour formation. Kidney and stomach tumours are clearly found at 175 mg/kg bw per day. Slight increase in kidney tubular adenomas and non-glandular stomach carcinomas occur at 15 mg/kg bw per day. A NOAEL based upon non neoplastic lesions (indicative of the mechanism of action for tumourgenicity) is 1.8 mg/kg bw/day from the long term rat study. Although there are indications that man is less susceptible than the rat a prudent approach is to base the ADI on the results of the rat studies.

2.9. Since the effect is associated with carcinogenicity a safety factor of 200 is applied to the NOAEL of 1.8 mg/kg bw/day giving an ADI of 0.01 mg/kg bw. When the lower susceptibility of man compared to the rat is completely proven a conventional safety factor of 100 could be used.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF HBNs

(Opinion expressed by the SCP on 11 February 1992)

BACKGROUND AND TERMS OF REFERENCE

Hydroxybenzonitriles (HBNs) have been used as herbicides for more than 20 years. The main representatives of this group are bromoxynil, ioxynil and their salts and esters.

Bromoxynil and ioxynil are the common names for 4-hydroxy-3,5-dibromo-benzonitrile and for 4-hydroxy-3,5-diiodobenzonitrile, respectively.

They are contact herbicides with some systemic activity, used in most Member States to control broad leaved weeds in maize (especially bromoxynil), cereal crops (mainly ioxynil), sorghum, onions, garlic and other crops. They inhibit photosynthesis by uncoupling oxidative phosphorylation. Both of the compounds and their derivatives are decomposed rapidly in soils (half-life 10 days). Their solubility in water is 130 mg/l (bromoxynil) and 50 mg/l (ioxynil).

The main concern for these compounds relates to possible developmental effects and for this reason the Commission decided to request the Scientific Committee for Pesticides to examine their toxicology and to give an opinion on the following questions:

Is the use of HBNs, in accordance with good agricultural practice, prejudicial to human health or the environment and if so, can such dangers be eliminated by reduction of potential exposure?

1. DISCUSSION - TOXICOLOGICAL ASPECTS OF BROMOXYNIL

1.1. Penetration, Metabolism and Excretion

^{14}C-Bromoxynil octanoate has been administered to rats by the oral route. In male rats ca. 87% was excreted via the urine and 3,6% via the faeces. In females 80 and 5,4% were excreted via urine and faeces, respectively. The T1/2 for urinary excretion was approximately 25 and approximately 50 hours for males and females, respectively. Elimination from plasma occurred with half -lives of approximately 40-50 hours. After 7 days 3 and 6% of the dose was retained in the body for males and females, respectively. Highest concentrations were found in plasma, liver, kidneys and in females in the thyroid. In the urine 60% of the dose was present as free bromoxynil and 13% as bromoxynil conjugates, while in the faeces bromoxynil octanoate and free bromoxynil were present. In plasma bromoxynil was bound to plasma proteins which may explain the relatively long half-life of bromoxynil in plasma (1, 2).
In a dermal experiment ^{14}C-bromoxynil phenol has been applied to the skin of male rats. After 10 hours the highest percentage (90-95%) was recovered in the skin wash. One to 2% of the dose remained in the body and 0,2-0,3% was excreted in the urine. Systemic absorption varied from 5-10%, inversely related to the dose. (3)
In vitro absorption with the formulation Advance (containing bromoxynil octanoate and ioxynil octanoate, together approximately 185 g/l) was studied in human and rat epidermis. It was found that absorption by rat skin was higher than by human epidermis, but no factor for the difference can be given (4).The formulation Oxytril (containing the same substances with a total amount of approximately 580 g/l) was only studied in human epidermis. The data obtained indicated that both substances are slowly absorbed across human epidermis (5).

1.2 BROMOXYNIL

1.2.1 Acute/short term toxicity

The oral LD_{50} in the rat for bromoxynil is approximately 190 mg/kg bw and higher than 2000 mg/kg bw by the dermal route. The LC_{50} for inhalation (4 hours) is approximately 0.4 mg/l air. Bromoxynil and the octanoate ester are not irritating to the skin and slightly irritating to the eye.
In short term toxicity studies in rats the main effects were growth inhibition, an increase in red blood cell parameters (Hb, Ht, RBC), urea and SAP activity in blood, a decrease in glucose and thyroid

hormones in blood and an increase in liver, kidney and thyroid weight. Histopathological effects were observed in liver and kidney. These effects are consistent with the uncoupling effects of bromoxynil on oxidative phosphorylation. In short term dog studies a decreased weight gain, an increased urea content in blood and an increased liver weight were also observed, but red blood cell parameters were decreased (see table 1).

1.2.2 Long term toxicity

Two long term studies in the rat and one in the mouse are available (see table 1).
In the rat study with the highest dose levels the same effects were observed as in the short term studies in the rat. In the other only an increased liver and kidney weight was found. Both rat studies did not show an increased tumour incidence. In the mouse study liver and kidney weights were increased. Male animals showed a slightly increased incidence of liver adenomas and carcinomas. The combined incidences were 3, 7, 8 and 15% for 0, 10, 30 and 100 ppm, respectively.

1.2.3 Mutagenicity

Bromoxynil was not mutagenic in various Ames tests (18, 19, 20), a gene mutation test in V79 cells (21) and a chromosome aberration test in human lymphocytes (22). Positive effects were obtained in a mouse lymphoma test (possibly due to a pH-effect) and a chromosome aberration test in CHO cells, but only after metabolic activation (23, 24). An SCE test in CHO cells was negative (25).
Negative results were obtained in a dominant lethal test in rats (26) and 3 micronucleus tests in mice (27, 28, 29). A chromosome aberration test in Chinese hamsters gave equivocal results (31). Negative results were also obtained in a UDS test in rat liver cells (32) and a transformation test in mice (33). A DNA-repair test in E coli was positive (34).

1.2.4 Developmental toxicity

1.2.4.1. Reproduction toxicity

Two reproduction studies have been carried out in rats (see table 1). The main effects were decreased maternal body weight and pup weight, with in one study a decrease in litter size and pup viability and in the other a delayed pup development, both at the highest dose level.

1.2.4.2. Teratogenicity

Nine studies have been performed with bromoxynil on rats and rabbits by the oral or percutaneous routes.
Two general reviews have been carried out by J.C. Lamb & B. Neal (35) and by E.M. Johnson & M.S. Christian (36). The main studies are summarized as follows:

- RATS experiments by oral route.

Three gavage studies on bromoxynil phenol were carried out in rats (37, 38, 39). The doses used and the main observations reported in these studies are summarized in table 2.

At the highest dose level (35-40 mg/kg bw), a number of embryotoxic and teratogenic effects are present at a maternal toxic dose level (mortality, effect on growth and food consumption)
Besides litter loss, post-implantation loss, fetal loss, reduced fetal weight, extra ribs and delayed ossification irreversible structural defects were present (an/microphthalmia).
At 12,5-15 mg/kg bw the same maternal toxic and embryotoxic effects were present, but there were no irreversible structural abnormalities. At 5 mg/kg bw slight embryotoxic effects were present. Although at 4 mg/kg bw extra ribs were found, this is considered as a marginal effect level. The NOAEL for maternal toxicity is 5 mg/kg bw.
A clear NOAEL cannot be established in the experiment (a) (Ref. 37) using doses of 1.5, 5 and 15 mg/kg/day. However, this study did not demonstrate statistically significant effects in either dams or offspring at any dose tested. In addition, this study, prior to GLP, had a weak study design.

- RABBITS experiments by oral route.

Two gavage studies on bromoxynil phenol were carried out in rabbits (40, 41). The doses used and the main observations reported in these studies are summarized in table 3.

At the highest level (45-60 mg/kg/day) embryotoxic effects were seen (increased post-implantation loss, increased incidence of fused and supernumerary ribs) along with evidence of teratogenicity (hydrocephaly and microphthalmia/anophthalmia).
The dose of 15 mg/kg/day, according to the extra ribs and the slight decrease in fetal weight can be regarded as a marginal effect level.
Maternal toxicity is demonstrated by a slightly reduced weight gain and food intake occurring at 30 and 45 mg/kg/day and mortality at 60 mg/kg/day. The NOAEL for maternal toxicity in rabbits can be estimated at 15 mg/kg/day.

- RATS experiments by dermal route.

Two dermal studies with bromoxynil phenol and bromoxynil octanoate were carried out in rats. The doses used in these studies were: 0,5, 10, 50 and 100 mg/kg/day in the first study (42) and 0, 2, 5, 10, 15, 20 and 75 mg/kg/day in the second one (43). The main observations from these studies are reported below.

Embryotoxicity: Using bromoxynil-phenol, an increased incidence of supernumerary ribs was reported at 50 and 100 mg/kg/day. With 75 mg/kg bw bromoxynil octanoate mean fetal body weight was decreased and the incidence of supernumerary ribs was increased. This last effect was also observed at 20 mg/kg bw.

From both studies it can be concluded that the NOAEL for embryotoxicity is 15 mg/kg bw/day.

Maternal toxicity: The maternal weight gain was decreased at 50 mg/kg/day and above (decreased food consumption was evident for the highest dose) for bromoxynil-phenol and at 20 and 75 mg/kg/day for bromoxynil octanoate. In the latter case, erythema and fissuring of skin were also observed.

The NOAEL for maternal toxicity of bromoxynil phenol and bromoxynil octanoate administered by dermal route in rats can be estimated at 15 mg/kg/day.

- RABBITS experiments by dermal route.

Two dermal studies were carried out in rabbits. Bromoxynil phenol was used in the first study (44) at 0, 10, 50 and 150 mg/kg/day, bromoxynil octanoate in the second (45) at 0, 5, 10, 15, 20, 40 and 80 mg/kg/day.

Embryotoxicity: An increased incidence of agenesis of intermediate lobe of lung at 50 and 150 mg bromoxynil phenol/kg/day is considered to be an incidental finding not related to treatment.

No dosage-dependent increased incidence of malformations or variations has occurred with bromoxynil octanoate. The NOAEL is higher than 80 mg/kg/day for octanoate and >150 mg/kg for phenol.

Maternal toxicity: With bromoxynil-phenol, maternal weight gain and food consumption are decreased at 50 mg/kg/day and above. The NOAEL is 10 mg/kg/day.

With bromoxynil octanoate, skin irritation is observed from 20 mg/kg/day and decreased maternal weight gain and food consumption are observed from 40 mg/kg/day. The NOAEL for systemic effects may be estimated at 20 mg/kg/day.

From both studies, it can be concluded that 20 mg/kg bw/day is the NOAEL for maternal toxicity.

1.3 IOXYNIL

1.3.1. Acute/short term toxicity

The oral LD_{50} in the rat for ioxynil is ca. 110 mg/kg b.w. and 1050 mg/kg b.w. by the dermal route. The LC_{50} for inhalation (4 hours) is ca. 0,4 mg/l air. Ioxynil and the octanoate ester are not irritating to the skin and only very slightly irritating to the eye. The sodium salt, however, is strongly irritating.

In short term studies in rats the main effects were growth inhibition and an increased liver and kidney weight (see table 4).

1.3.2 Long term toxicity

Chronic/carcinogenicity studies have been carried out in mice and rats (see table 4).
In rats an increased thyroid weight, histopathological abnormalities and an increased incidence of thyroid tumours were found at 30 and 100 ppm. The thyroid tumours are associated with the hyperplastic effects caused by ioxynil on the thyroids.
At 10 ppm (equivalent to 0.5 mg/kg bw.) no effects were observed.
In mice mortality was increased at 100 ppm . At all dose levels body weight gain was lower and relative weights of kidneys and thyroid were increased. Liver weight was increased at 30 and 100 ppm. At 100 ppm a slightly higher incidence of liver tumours (hepatocellular adenomas and carcinomas) was observed in male animals. The combined incidence was 5% for control animals and 15% for high dose animals.

1.3.3 Mutagenicity

Ioxynil was not mutagenic in various Ames test (52, 53, 54, 55) and with mutagenicity tests in Saccharomyces cerevisiae (56) and Aspergillus nidulans (57). In two mouse lymphoma tests (58, 59) equivocal results were observed. The same was found in a chromosomal aberration test in human lymphocytes (only without metabolic activation) (60), but an SCE-test in human lymphocytes was negative (61).
Dominant lethal tests in rats (62) and mice (63) and two micronucleus tests in mice (64, 65) were negative. An UDS-test in rats hepatocytes (66) and two bacterial tests for DNA repair were also negative (67, 68).

1.3.4 Developmental toxicity

1.3.4.1. Reproduction toxicity

In a 3-generation reproduction study in the rat a decreased maternal body weight gain was observed at all dose levels. At 100 and 300 ppm litter size and pup weight were decreased. At these dose levels also liver and thyroid weight were increased (see table 4).

1.3.4.2 Teratogenicity

- Rats and rabbits experiments by oral route.

Three gavage studies on loxynil phenol were carried out in rats (69, 70, 71) and one in rabbit (72). The doses used and the main observations reported in these studies are summarized in Table 5 and 6 for rats and rabbits, respectively.
From the results obtained in the rat studies the following can be concluded.
At the highest dose level of 35-36 mg/kg bw a number of embryotoxic and teratogenic effects were present at a maternal toxic dose level (mortality, effect on growth and food consumption). Besides litter loss, post-implantation loss, fetal loss, reduced fetal weight, extra ribs, hydroureters and minor abnormalities, irreversible structural defects were present (an/microphthalmia) at 36 mg/kg bw. At 12-15 mg/kg bw some embryotoxic effects were present but no irreversible structural abnormalities. A dose of 5 mg/kg bw can be considered as an NOAEL for embryotoxicity and 12-15 mg/kg bw for maternal toxicity.

In the rabbit study (72) the doses administered were 0 - 15 - 30 and 60 mg/kg bw/day. The results are summarized in table 6.
Maternal toxicity was observed at 60 mg/kg bw (mortality, effect on growth and food consumption). At 30 mg/kg bw only effects on growth and food consumption were present. At both dose levels litter loss, post-implantation loss, reduced fetal weight, extra ribs, rib malformations, hydrocephaly and an/microphthalmia were present. loxynil shows therefore irreversible structural abnormalities. At 15 mg/kg bw only one animal showed hydrocephaly. This dose level can be considered as a marginal effect level. The NOAEL for maternal toxicity is 15 mg/kg bw.

- Rats and rabbits experiments by dermal route.

Two dermal studies with ioxynil phenol were carried out, one in rats with doses of 0, 5, 10, 40 and 120 mg/kg bw/day (73) and one in rabbits with doses of 0, 10, 50 and 150 mg/kg bw/day (74).

- rat study

Decreased maternal body weight gain was observed at 40 and 120 mg/kg bw and reduced food consumption from 10 mg/kg bw on. Skin reactions were present at the highest dose level. At 120 mg/kg b.w. the incidence of supernumerary ribs and wavy ribs was increased. The NOAEL for embryotoxicity is 40 mg/kg bw. For maternal toxicity 10 mg/kg bw can be considered as the NOAEL.

- rabbit study

At 150 mg/kg bw only maternal body weight gain and food consumption were decreased. NOAEL's for embryotoxicity and maternal toxicity are > 150 and 50 mg/kg bw, respectively.

2. HUMAN DATA – OCCUPATIONAL EXPOSURE

Four studies have been carried out in USA (76, 77), UK (78) and Canada (79) to assess the degree of contamination of workers during the application of HBN formulations. The main observations and the conclusions of these studies are summarized below and in Table 7.

2.1. USA (BUCTRIL = Bromoxynil octanoate)

In the 1988 (76) four workers experienced in mixing, loading, application, and clean up procedures of pesticides were selected to participate in the exposure study. Buctril was applied to a mint field and a barley field as recommended in practice. Two work days were monitored.

Airborne exposure was measured with a sampling system. Dermal exposure assessment was evaluated with gauze-pad dosimeters located underneath and on the outside of coveralls. Hands were washed with a detergent solution at the end of each work cycle to assess dermal exposure of the hands. Urinary samples were taken for the metabolic study.

Mixer-loaders handled, without gloves, approximately 15 kg of bromoxynil per day. Almost all the exposure was to the hands. The total dermal exposure during mixing/loading without gloves can be estimated between 0.8 and 1.3 mg/kg bw/day. During application, exposure was about 0.05 mg/kg/day. The levels of bromoxynil in the urine were negligible.

The 1990 study (77), was conducted at 20 sites throughout the United States where corn, wheat and sorghum are grown. Buctril, utilizing revised container designs and up-to-date application technology, was applied at the highest rate for these crops (420 g/ha). At each site, a mixer/loader and an applicator volunteered to participate to the study and two replicates were conducted. The sprays were made with enclosed or open cabin equipment. Both inhalation and dermal exposure were monitored and potential and actual exposure were calculated for protected and not protected exposures (enclosed or open cabin, gloves or not in the open cabin).

During mixing/loading dermal exposure was estimated at 0.004 mg/kg bw/day.
During application dermal exposure in the enclosed cabin was negligible. Dermal exposure (open cabin) was estimated at 0.003 mg/kg bw/day without gloves and 0.002 with gloves.

2.2. UK (OXYTRIL CM = Bromoxynil octanoate and Ioxynil octanoate 200 g/l each).

In a 1989 study by Lamb et al. (78) (which also reviews dermal penetration and worker exposure), four workers were examined during the application of oxytril to 20 hectares of land, using equipment and clothing typical of UK farming practice. The degree of contamination of coveralls, gloves, air, hands, inner clothing, blood and urine was monitored.

As with external contamination the hands received the bulk (60 %) of the exposure. Inhalation was not found to be a significant route of exposure. Levels of bromoxynil and ioxynil in plasma and urine were extremely low.

Mixer-loaders, wearing gloves, handled about 32 kg of bromoxynil + ioxynil per day.. The overall dermal exposure of workers was estimated at 0.013 mg/kg bw/day.

2.3. CANADA (BUCTRIL M = bromoxynil as octanoate and heptanoate esters + MCPA as iso-octyl ester 200 g/l each).

In 1988 an exposure study was conducted at five farm locations in Manitoba (79). Buctril M was applied at the recommended rate of 0.56 kg active ingredient per hectare. Mixer-loaders handled approximately 18 kg of bromoxynil per day. Because the actual field-use conditions were so variable, the resulting exposure data showed great variability.

Bromoxynil recovered from hand washes ranged from 0 to 650 μg/hr. This exposure was significantly influenced by wearing protective gloves (273 - 657 μg/hr without gloves, 0- 114 μg/hr with gloves).

The dermal exposure of these workers may be estimated at 0.02 mg/kg bw/day with gloves and 0.05 without them.

2.4. Skin penetration experiments.

Comparing the in vivo skin penetration experiments in rats and the in vitro comparison between rats and human skin, the following results are obtained. In the rat in vivo, dermal penetrations of bromoxynil and ioxynil are comparable. The absorption rates for bromoxynil phenol, bromoxynil octanoate and ioxynil in rats were all about 1 % of the applied dose per hour. This corresponds to a 10 % exposure dose during a 10-hour work/day. According to three in vitro tests, HBN penetration through human skin seems to be lower than through rat skin (4)(5).

2.5. Comments

2.5.1 Discrepancies are naturally found between the four studies, due partly to the ways of handling the formulations in the different countries and partly to the assumptions and the conditions of sampling for exposure. Only orders of magnitude have to be taken into account.

2.5.2 Skin surface contamination is the main route of exposure. Respiratory exposure is very low (approximately 3.5 % of total contamination). Levels of bromoxynil and ioxynil in plasma and urine are also very low, indicating that in practice very little is absorbed.

2.5.3 Mixer-loaders are much more exposed than the applicators. Gloves insured a very good protection and in the second US study it was established that special containers resulted in a lower exposure.

3. ENVIRONMENTAL ASPECTS

3.1. Aquatic toxicity

3.1.1. Algae.

Bromoxynil phenol was tested for toxicity to the green fresh-water alga Scenedesmus subspicatus. The EC50 (growth rate inhibition 96 h) was 140 mg/l and the EC50 (plateau growth level inhibition 96 h.) was 44 mg/l.

3.1.2. Daphnia.

Bromoxynil phenol was tested for toxicity to daphnia magna. The EC50 (24h or 48h) was 12.5 mg/l (79).
Bromoxynil octanoate in identical conditions was much more toxic, the EC50 (48h) being 0.11 mg/l (80).
Daphnia were continuously exposed to measured bromoxynil octanoate concentrations ranging from 0.91 to 24 µg/l through one generation. The estimated (NOAEL) after 21 days exposure was found to be between 2.6 and 5.3 µg/l (81).

3.1.3. Fish.

The LC_{50} for fish are the following:
Bromoxynil phenol (96h) : Rainbow trout = 23 mg/l (82). The substance was used after neutralization with KOH. It has to be noted that the aquatic toxicity of bromoxynil phenol is mostly depending on the pH of the water ranging from 0.2 mg/l at pH 6.2 to 20 mg/l at pH 8.2 .
Bromoxynil octanoate(96h): Rainbow trout = 0.1 mg/l, Bluegill = 0.061 mg/l (83)
loxynil octanoate(48h): Harlequin fish = 4 mg/l

3.1.4. Effect on early stage aquatic organisms.

Fathead minnow (Pimephales promelas) embryos and larvae were continuously exposed for 35 days to nominal concentrations of 3, 6, 12, 25 and 50 µg/l Bromoxynil octanoate in pH 7.1-7.6 ketone -water solutions. The most sensitive indicator of toxicity was larval survival. According to the analytical doses verification, the NOAEL was estimated to be between 9 and 18 µg /l (84).

3.2. Toxicity to birds.

The acute oral LD_{50}'s of bromoxynil to avian species range from 50 (bromoxynil-phenol and bromoxynil-K salt, pheasant) to 240 mg/kg bw (bromoxynil-phenol, hen). The LD_{50}'s for ioxynil range from 35 (ioxynil-Na salt, pheasant) to over 1200 mg/kg bw (ioxynil-octanoate, Mallard duck).
The most sensitive species appears to be the pheasant. The approximate acute oral LD_{50}'s (mg/kg) for this species are summarized in Table 8.

3.3. Effects on soil and soil organisms.

3.3.1. Microorganisms.

Ioxynil and bromoxynil are fairly inhibitory to fungi and bacteria at low concentrations but these concentrations are far in excess of what are likely to be found in soil during current agricultural practice.

3.3.2. Earthworms.

An artificial soil test was done in accordance with OECD protocols to investigate the toxicity of ioxynil to Eisenia foetida. The LC_{50} (7 and 14 days) was approximately 35 mg/kg soil (85).

3.4. Leaching.

The leaching of ioxynil-ring ^{14}C has been studied in sand, loam, peat and clay soils in laboratory tests. Data indicate that ioxynil should demonstrate low mobility when freshly applied in solution to these soil types. Absorption appears to increase with increasing organic matter content (37).

3.5. Soil degradation.

The rate of degradation of radio-labelled ioxynil has been studied in sand, loam, clay and organic loam soils (peat). The half-lives of both extractible herbicide-derived material (iodo-hydroxybenzamide) and ioxynil were greatest in the peat (75 days at 10°C , 15 to 23 days at 20°C). In the other soils the degradation was much faster (3 to 10 days at 10°C and 1.5 to 3.5 at 20°C).

4. GENERAL CONCLUSIONS

4.1 Hydroxybenzonitrile (HBN) products are widely used in Europe and sold currently in 68 countries in the world. They are used primarily in cereal crops including maize and in onions. They are associated with many other herbicides in formulations. Bromoxynil and ioxynil are the most representative substances of the series.

4.2 In general the toxicological profiles for bromoxynil and ioxynil are comparable. Both compounds are uncouplers of oxidative phosphorylation. Effects of this are more clearly demonstrated for bromoxynil, for which more toxicity data are available. In general a decreased body weight gain and an increased liver and kidney weight are observed as the most sensitive parameters. An increased thyroid weight is more prominently found with ioxynil, possibly because of the high iodine content of the compound, although this effects is also found with bromoxynil. With both compounds a slightly increased incidence of liver tumours is found in male mice, at the highest dose level. An increase in thyroid tumours is found in the long term rat ioxynil study. Since these tumours are related to the toxicity effects on these organs and mutagenicity data are generally negative the compounds are considered not to be genotoxic substances and not to present a carcinogenic risk to man. From the short term, long term and reproduction toxicity experiments with bromoxynil an overall NOAEL of 1 mg/kg bw is chosen based on a short term study in dogs.

For ioxynil the overall NOAEL of 0,5 mg/kg bw is based on the long term rat study. Although lower NOAEL's were derived for individual studies, the selected doses are the highest NOAEL's which are still lower than the actual adverse effect levels from all studies. Based on these NOAEL's ADI's of 0.01 and 0.005 mg/kg bw can be estimated for bromoxynil and ioxynil, respectively.

4.3 Teratogenic and embryotoxic effects for both compounds are found at higher dose levels than for general toxicity. However, these effects may be of more concern for the evaluation of the risk for occupationally exposed people.

Teratogenic effect are only found by the oral route at high,

maternally toxic, dose levels both in rats and rabbits. Embryotoxic effects are found at lower levels, which are sometimes not maternally toxic. Dermal application gave embryotoxic effects at high dose levels. In this respect the rat was the most sensitive species. The NOAEL's for maternal toxicity are based on growth inhibition, which is also one of the most sensitive parameters in the short and long term toxicity experiments.
NOAEL's for developmental effects are summarized in table 9.

Table 9: Maternal and fetotoxicity NOAELs (mg/kg/day) for bromoxynil and ioxynil.

	RATS		RABBITS	
	fetal	maternal	fetal	maternal
BROMOXYNIL				
oral route	4*	5	15*	15
dermal route	15	15	>80	>20
IOXYNIL				
oral route	5	15	15*	15
dermal route	40	10	>150	50

*: marginal effects

Because the main exposure for application is dermal, NOAEL's for maternal toxicity by the dermal route are chosen to calculate the maximum allowable exposure for operators.
For bromoxynil and ioxynil the NOAEL's are 15 and 10 mg/kg bw/day respectively. Taking into account a safety margin of 100 the maximum allowable exposure is 0.15 mg/kg bw/day for bromoxynil and 0.10 for ioxynil.

4.4 Application of bromoxynil and ioxynil is generally in such a way that no residues in crops are to be expected. Therefore when consumer exposure is compared to the ADI, the risk for consumers is very limited.
A higher risk is present for applicators. For unprotected workers (without gloves) the dermal exposure is approximately 1 mg/kg bw/day which is higher than the maximum allowable exposure derived in paragraph 4.3. For protected workers, however, with a maximum exposure of 0.02 mg/kg bw/day the margin of safety is acceptable.
Therefore it should be strongly recommended that protective gloves should always be used and the other protective measures, such as specially adapted containers, should be introduced to reduce exposure.
Safety margins will be even more favourable since, from the limited studies available, it can be concluded that penetration through human epidermis is lower than through rat skin.

4.5 From ecotoxicological data it can be concluded that bromoxynil and ioxynil phenol are moderately to very toxic to Daphnia and fish, strongly depending on the pH of the test solution. Bromoxynil octanoate is very toxic. HBN's have a short half life and a low mobility in soil. HBN's should be used with care to avoid contamination of water, so that exposure to aquatic life is minimized.

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Table 1: Short term, long term and reproduction toxicity studies with bromoxynil

	dose levels	NOAEL	Effects	Ref
Short term rat (1) (90 d)	0-20-50-125-312 -781-1953 ppm	125 ppm eq. to 6.25mg/kg bw/d	decr. body wt, incr. red blood cells, incr. urea, incr. liver/kidney wt	6
Short term rat (2) (90 d)	0-800-1200-1600 ppm and higher	< 800 ppm eq. to < 40 mg/kg bw/d	mortality (1200 ppm and higher), decr. body wt, incr. red blood cells, urea, SAP, decr. glucose T3/T4, incr. liver/ kidney/thyroid wt, histopath. liver, thyroid.	7
Short term rat (3) (90 d)	0-30-150-600 ppm	30 ppm eq. to 1.5 mg/kg bw/d	decr. body wt, incr. red blood cells, urea SAP decr. glucose, incr. liver/kidney wt	8
Short term rat (4) (90 d)	0-150-400-950 ppm	< 150 ppm eq. to <7.5 mg/kg bw/d	decr. body wt, incr. red blood cells, urea, SAP decr. glucose, incr. liver/ kidney/thyroid wt., liver/histopath.	9
Short term rat (5) (90 d)	0-10-50-400-800 ppm	10 ppm eq. to 0.5 mg/kg bw/d	decr. body wt, incr. red blood cells, urea, SAP, decr. T3/T4, incr. liver/ kidney/thyroid wt., liver/thyroid histop.	10
Short term dog (1) (90 d)	0-1-5-25 mg/kg	1 mg/kg/bw/d	decr. body wt, decr. red blood cells, incr. urea, incr. liver wt.	11

Short term dog (2) (1 yr)	0-0.1-0.3-1.5 7.5 mg/kg bw/d	0.3 mg/kg bw/d	decr. body wt, decr. red blood cells, SAP, incr. glucose, urea, incr. liver wt.	12
Long term rat (1) (2 yr)	0-10-30-100 ppm	30 ppm eq. to 1.5 mg/kg bw/d	decr. spleen wt, liver/kidney wt (no tumours)	13
Long term rat (2) (2 yr)	0-60-190-600 ppm	60 ppm eq. to 3 mg/kg bw/d	decr. body wt, incr. red blood cells, decr. glucose, incr. liver wt, liver histopathology (no tumours)	14
Long term mouse (18 mo)	0-10-30-100 ppm	10 ppm eq. to 1.4 mg/kg bw/d	incr. liver/kidney wt, incr. liver tumours (males)	15
Reproduction rat (1) (3 gen)	0-30-100-300 ppm	30 ppm eq. to 1.5 mg/kg bw/d	decr. body wt, decr. litter size, decr. pup wt, lower viability	16
Reproduction rat (2) (2 gen)	0-10-50-250 ppm	50 ppm eq. to 2.5mg/kg bw/d	decr. body wt, decr. pup wt, delayed eye opening, incr. liver/ kidney wt	17

Table 2: Teratogenic and embryotoxic effects of Bromoxynil phenol in rats by oral route.

Effects		Dose in mg/kg b.w./day							ref
	a --> 0	1.5		5		15			37
	b --> 0			5		15	35		38
	c --> 0		4		12.5			40	39
Litter loss	a	N	N		N		N		
	b	N			N		+*	+*	
	c	N		N		N			N
Post-implant loss	a	N	N		N		N		
	b	N			N		+	+	
	c	N		N		+			+*
Fetal loss	a	N	+		+		+		
	b	N			N		+*	+*	
	c	N		N		+			+
Fetal weight	a	N			N		N		
	b	N			N		N	_*	
	c	N		N		N			_*
Extra ribs	a	N	N		N		+		
	b	N			+		+*	+*	
	c	N		+		+*			+*
Wavy ribs	a	N	+		+		+		
Delayed ossification	b	N			+		+	+	
	c	N		N		+*			+*
An/Microph.	b	N			N		N	+	
	c	N		N		N			+*

* = statistically significant; N = normal; + = increased; - = decreased.

Table 3: Teratogenic and embryotoxic effects of bromoxynil phenol in rabbits by oral route.

Effects		Dose in mg/kg b.w./day					ref
	a -->	0	15	30		60	40
	b -->	0		30	45	60	41
Litter loss	a	N	N	N		+*	
	b	N		N	N	+*	
Post-implant loss	a	N	N	+		+*	
	b	N		N	N	N	
Fetal weight	a	N	–	–		–*	
	b	N		–	–	–*	
Extra ribs	a	N	+	+		+*	
	b	N		N	+	+	
Fused ribs	b	N		N	+	+	
An/Micropht.	a	N	N	N		+*	
	b	N		N	+	+	
Hydrocephaly	a	N	N	N		+*	
	b	N		N	+	+	
Minor abnormalities	a	N	N	N		+*	
	b	N		+	+*	+*	

* = statistically significant; N=normal; + = increased; – = decreased.

Table 4: Short term, long term and reproduction toxicity studies with ioxynil.

	dose levels	NOAEL	Effects	Ref
Short term (1) rat (90 d)	0-20-80-320 ppm	<20 ppm eq. to <1 mg/kg bw/d	decr. body weight, incr. liver/thyroid wt	46
Short term (2) rat (90 d)	0-12-37-111-333-1000-3000 ppm	37 ppm eq. to 2 mg/kg bw/d	mortality, decr. b.w., incr. liver wt (thyroid wt not measured)	47
Long term rat (2 yr)	0-10-30-100 ppm	10 ppm eq. to 0.5 mg/kg bw/d	incr. liver/kidney/ thyroid wt, thyroid tumours	48
Long term mouse (18 mo)	0-10-30-100 ppm	< 10 ppm eq. to < 1.4 mg/kg bw/d	decr.body wt., incr. liver/kidney/ thyroid wt, incr. liver tumours (males)	49/50
reproduction rat (3 gen.)	0-30-100-300 ppm	< 30 ppm eq. to <1.5 mg/kg bw/d	decr.body wt., reduced litter size, reduced pup wt, incr. liver/ thyroid wt.	51

Table 5: Teratogenicity and embryotoxic effects of ioxynil phenol in rats by oral route.

Effects		Dose in mg/kg b.w./day						ref
	a --> 0			5	15	35		69
	b --> 0					35		70
	c --> 0		4	12			36	71
Litter loss	a	N		N	N	+		
	b	N				N		
	c	N	N	N			N	
Post-implant loss	a	N		N	N	N		
	b	N				N		
	c	N	N	N			+*	
Fetal loss	a	N		N	N	+		
	b	N				N		
	c	N	N	N			+	
Fetal weight	a	N		N	N	_*		
	b	N				_*		
	c	N	N	_*			_*	
Extra ribs	a	N		+	+*	+*		
	b	N				+*		
	c	N	N	N			+*	
Minor abnormalities	a	N		N	+	+*		
	b	N				+*		
	c	N	+	+			+*	
Hydroueters	a	N		N	N	+*		
	b	N				+*		
An/microph.	a	N		N	N	N		
	b	N				N		
	c	N	N	N			+*	

* = statistically significant; N=normal; + = increased; - = decreased.Table

Table 6: Effect of ioxynil phenol in rabbits by oral route (72).

Effects	Dose in mg/kg/ bw/day			
	0	15	30	60
Litter loss	N	N	+	+
Post-implant. loss	N	N	+*	+*
Fetal weight	N	N	–	–
Extra ribs	N	N	+	+*
Rib malformations	N	N	+	+*
An/Microphta	N	N	+	+*
Hydrocephaly	N	+	+	+*

* = statistically significant; N=normal; + = increased; – = decreased.

Table 7: Exposure of workers in different mixing-loading situations.

(Refs)	USA bromoxynil octanoate (76)	USA bromoxynil octanoate (77)	UK brom.oct +ioxynil (78)	CANADA brom. oct. +ioxynil+MCPA (79)
Quantity handled (kg/day)	5	17	32	18
Total dermal exposure mixer-loaders				
without gloves ..	0.8-1.3	-	-	0.05
with gloves	-	0.004	0.013	0.02
(mg/kg bw/day)				

Table 8: Acute oral LD_{50}'s of Ioxynil and Bromoxynil derivatives for pheasant.

Formulation	Ioxynil (mg/kg)	Bromoxynil (mg/kg)
Phenol	75	35
K salt	35	50
Octanoate	1000	175

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF CHLORPYRIFOS-METHYL

(Opinion expressed by the SCP on 2 June 1992)

BACKGROUND AND TERMS OF REFERENCE

Chlorpyrifos-methyl is an organophosphate insecticide and acaricide for grain store use which was discussed at the JMPR meeting in Geneva in September 1991. The WHO panel of experts reviewed the available toxicology data and the Meeting concluded that the ADI should be reduced by a factor of 10 from 0.01 mg/kg bw to 0.001 mg/kg bw.

After the meeting the major registrant of chlorpyrifos-methyl products (Dow-Elanco) stated that they did not agree with the conclusions of the JMPR meeting regarding the ADI for chlorpyrifos-methyl. Further data were submitted and considered by the JMPR meeting in Rome in September 1992. At that meeting the JMPR ADI was returned to its original value of 0.01 mg/kg bw.

In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the basis of the ADI, paying particular attention to the data considered by the JMPR in 1991 and 1992.

1. ORIGINAL JMPR ADI

In 1975 the JMPR meeting reviewed the available toxicology data on chlorpyrifos-methyl and allocated an ADI of 0.01 mg/kg bw. The most critical study leading to this conclusion was a human volunteer study in which groups of 5 males were given doses of 0.03 or 0.1 mg/kg bw/day for 4 weeks. Treated subjects remained comparable to 4 male control subjects with respect to plasma and erythrocyte cholinesterase activity, and other laboratory and clinical investigations. The NOAEL was therefore the highest dose tested, 0.1 mg/kg bw, and the ADI was allocated using a 10 fold safety factor. (Ref. 1)

2. JMPR MEETING IN 1991

2.1. EVALUATION OF DATA

Acute toxicity studies, short-term studies in mice, rats and dogs, long-term/ carcinogenicity studies in mice and rats, studies on delayed neurotoxicity, a teratogenicity study and a range of mutagenicity studies were submitted for evaluation by the 1991 JMPR meeting. These studies are briefly described below, along with the NOAEL's from each experiment and the adverse findings on which the NOAEL was based.

Evaluation of the acute toxicity data revealed that chlorpyrifos-methyl is moderately acutely toxic by the oral route (LD 50: 2680->5000 mg/kg bw). No specific signs of toxicity were observed and there was no significant difference between sexes. (Refs. 2, 3)

In a 28-day dietary study in mice (0, 1, 5, 10, 1000 and 10000 ppm) the NOAEL was 10 ppm, equal to 1.4 mg/kg bw/day based on brain cholinesterase inhibition and alterations in the adrenal glands at 1000 ppm. (Ref. 4)

In a 13-week dietary study in rats at constant doses of 0, 0.1, 1, 10, and 250 mg/kg bw/day the NOAEL was 1 mg/kg bw/day, based on histological alterations in the adrenal glands at 10 mg/kg bw/day. (Ref. 5)

In a 13-week dietary study in dogs at doses of 0, 0.1, 10 and 50 mg/kg bw/day, the NOAEL was 10 mg/kg bw/day based on inhibition of brain cholinesterase activity, increased liver weight and reduced body weight gain at 50 mg/kg bw/day. (Ref. 6)

In a 78-week study in mice using dietary concentrations of 0, 1, 5, 50 and 500 ppm, the NOAEL was 50 ppm, equal to 4 mg/kg bw/day. At 500 ppm, inhibition of brain cholinesterase activity was observed along with centrilobular hepatocyte fatty change and cortical cellular swelling in the adrenals. There was no treatment-related effect on the incidence of neoplastic changes. (Ref. 7)

In a 2-year dietary study in rats using dietary concentrations of 0, 1, 2, 20 and 1000 ppm, the NOAEL was considered to be 2 ppm, equal to 0.1 mg/kg bw/day. A slight increase in the incidence of adrenal cortical vacuolation compared to controls was seen at 20 ppm and a marked increase in incidence and severity was seen at 1000 ppm. Brain cholinesterase inhibition was consistently observed at 1000 ppm. There was no evidence that chlorpyrifos-methyl was carcinogenic in rats. (Ref. 8)

Chlorpyrifos methyl did not cause delayed neurotoxicity in hens. (Refs. 9, 10)

An oral teratology study in rabbits (0, 4, 8 and 16 mg/kg bw/day) was negative at all doses. (Ref. 11)

The results of a range of mutagenicity studies were examined (an Ames test, a CHO/HGPRT mutation assay, a chromosome aberration assay in CHO cells, a UDS assay in cultured rat liver cells and an in vivo mouse micronucleus test). It was concluded that chlorpyrifos-methyl was not genotoxic; the clastogenic response in the presence of metabolic activation was considered not to be of any relevance in the presence of a negative in vivo micronucleus test. (Refs. 12-16)

2.2. ESTIMATION OF ADI

The ADI was based on the results of the long-term rat study, using a 100 fold safety factor. Adrenal changes were seen consistently in rats and mice and the use of the human acetylcholinesterase inhibition data (reviewed by the JMPR in 1975) for ADI estimation was not possible because adverse effects on the adrenals were found in the rats in the absence of cholinesterase inhibition.

3. JMPR MEETING IN 1992

No new toxicological studies were submitted for evaluation at the 1992 JMPR meeting, but further, explanatory data were considered. The meeting re-evaluated the long-term rat dietary study on which the ADI was based in 1991. The major interest was the vacuolation of the adrenal gland. Interpretation of these data was aided by examination of relevant, contemporary control data from other rat studies performed in the same laboratory. (These background data were not supplied to the 1991 meeting) The incidence and severity of adrenal vacuolation at all dose levels except the high dose was within the control range. The historical control incidence ranged up to 42% in males and 10% in females, while the incidence in the control and lower dose groups was up to 20% in males and 4% in females. In the high dose group approximately 100% of animals were affected. The NOAEL for the long-term rat study was therefore set at 20 ppm, equal to 1 mg/kg bw/day. The ADI was revised accordingly, and based primarily on the human data. (Ref. 17)

4. OVERVIEW AND ESTIMATION OF ADI

The scientific committee endorsed the principles used by the JMPR in setting an ADI for chlorpyrifos-methyl.

In particular, the principles used in assessment of data relating to inhibition of cholinesterase are worthy of note. Inhibition of plasma (or 'pseudo') cholinesterase in animal studies is not considered to be an adverse effect useful in derivation of an ADI, but is a useful indicator of exposure. Inhibition of erythrocyte cholinesterase (a true acetylcholinesterase) is considered to be indicative of toxicity, but inhibition of around 25% compared to controls is required, before the effect is considered to be adverse. Inhibition of cholinesterase activity in brain is also considered to be indicative of toxicity, but in this case inhibition greater that 10% compared to controls

is considered adverse. When results from human volunteer studies are available these are generally used in risk evaluation in preference to results of animal studies, provided the human studies are of adequate quality and, most importantly, that the crucial parameters identified by the animal studies are investigated in the human volunteer studies.

In the case of chlorpyrifos-methyl, all the animal studies indicate that inhibition of acetylcholinesterase is the crucial paramenter. Adrenal effects were seen consistently in rats and mice, but the latest evaluation indicates that adrenal effects were only seen in the presence of cholinesterase inhibition. Allocation of the ADI may thus be based on the no adverse effect level for cholinesterase inhibition in the human volunteer study (0.1 mg/kg bw/day), utilising a 10 fold safety factor. The resultant ADI of 0.01 mg/kg bw is further supported by the no effect level from the rat study (1 mg/kg bw/day, based on cholinesterase inhibition and adrenal changes), and a 100 fold safety factor.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF ETHYLENE AND PROPYLENE BISDITHIOCARBAMATES AND THEIR METABOLITES / CONTAMINANT ETHYLENE THIOUREA (ETU) AND PROPYLENE THIOUREA (PTU)

(Opinion expressed by the SCP on 2 June 1992)

BACKGROUND AND TERMS OF REFERENCES

Ethylene bisdithiocarbamates (EBDC compounds) have been used as fungicides in agriculture in the Community since the early 1960s. Examination of the historical toxicological database reveals consistent thyroid effects and liver effects with all EBDCs. The reason for this common toxicity, ethylene thiourea has been identified since the early 1960s. ETU is a contaminant of EBDCs and metabolic product in both plants and animals. Crops treated with EBDCs have residues of ETU as well as the parent EBDC. While levels of parent compounds decrease during storage, processing or cooking, levels of ETU increase.

The current toxicological appreciation of EBDCs in Member States is based on the conclusion that all the relevant toxicology is related to ETU and directly associated with the effects of ETU on thyroid hormone homeostatis.

In the context of its work to establish maximum pesticide residue levels pursuant to the relevant Community legislation, the Commission invited the Scientific Committee for Pesticides to examine the toxicological data relating to the EBDCs and its metabolite ETU and to estimate their acceptable daily intake.

Propineb has a similar qualitative toxicity profile to the EBDCs and may have a similar relationship with PTU as that noted for ETU and EBDCs. Propineb and PTU are therefore dealt with in parallel with EBDCs and ETU.

1. TOXICOLOGICAL ASPECTS

1.1. Metabolism

Investigations of the in vivo metabolism of the ethylene bisdithiocarbamates (EBDCs) indicate that ethylene thiourea (ETU) is the major common metabolite responsible for the toxicity of the EBDCs [IPCS 1988 - (1)]. Data for maneb suggest that up to 13.6 % of a 25 mg/kg dose may be converted to ETU, in the case of metiram a conversion of up to 9.5 % of a 5 mg/kg dose has been reported whilst for zineb a conversion of 22 % of a 50 mg/kg dose has been recorded for the rat and marmoset. It can be concluded from the study by Nelson [1986 - (2)] that up to 23 % of a 100 mg/kg mancozeb dose can be converted to ETU in the rat. The figures for the conversion of EBDC's to ETU quoted above are all based on total ^{14}C with no conversion for relative molecular weights. Application of the conversion factor for the relative molecular weights of mancozeb and ETU shows that the maximum worst case in vivo conversion of EBDC's to ETU being no greater than of 10% on a weight for weight basis.

1.2. Sub-chronic studies - ETU

Thyroid and liver effects.

In a number of sub-chronic studies in rats (3-9), the thyroid has been identified as the target organ. Relevant findings are summarized in Table 1. The study by Freudenthal et al. (6) was chosen as the basis for establishing the NOAEL for the rat and it was established at 25 ppm equal to 1.78 mg/kg/day. The study of Leuschner (7) was not considered to be relevant for setting an overall NOAEL because the dose levels used were considered to be too low to see real effects of ETU and the findings were opposite to those expected for treatment with ETU. Effects on thyroid were also found in mice and monkeys (10, 11, 12). However it was difficult to establish NOAELs for these species because the two mouse studies gave quantitatively different results to one another. The monkey studies were unreliable because one was compromised by ill health of the animals whilst little reliance could be placed on the effects at the lowest dose used in the second(12).
In some of these studies in rats and mice, effects on the liver were also observed. These effects included increased liver weight, hepatocyte hypertrophy and induction of mixed function oxidases at relatively high dose levels compared to NOAELs based on thyroid effects.

1.3. Chronic studies - ETU

In a chronic study on rats by Graham et al. (1975) the thyroid was again the target organ (table 2). The conduct of the histological examination and the presentation of the results in the paper were not adequate to establish a NOAEL for thyroid hyperplasia. The incidence of thyroid follicular cell adenomas and carcinomas was increased at 250 and 500 and thyroid weights increased at 125 ppm and higher giving 25 ppm as the NOAEL equivalent to 1.25 mg/kg/day (13).

A further study by GAK et al. (1978) was not sufficiently well-supported to establish a NOAEL.

In a recent (1989) National Toxicology Programme (NTP) study on rats (15), which has not yet been fully reported, groups of 50 male and 50 female rats were exposed in utero to doses of 25, 83 and 250 ppm for 2 years. It was found that the incidence of thyroid tumours (follicular cell adenomas and adenocarcinomas) increased at 250 ppm, in males and females. There was also some indication of treatment related effects in males at 83 ppm.

In the NTP mouse feeding study of similar design, dietary levels of 0, 100, 333 and 1000 ppm were used in a 2 years carcinogenicity study (15). Thyroid tumours (follicular cell adenomas and adenocarcinomas) were observed at 1000 ppm in both sexes and in the females there was some indication of treatment related effects at 333 ppm. Liver tumours (hepatoblastomas and hepatocellular adenomas and carcinomas) were observed in a dose related manner at 333 and 1000 ppm in males and females.

From these studies it can be concluded that the NOAEL is 25 ppm for the rat (equivalent to 1.25 mg/kg bw) and 100 ppm for the mouse (equivalent to 15 mg/kg bw).

1.4. Sub-chronic studies - EBDCs

In a number of sub-chronic studies (11, 16-21) using different EBDCs (mancozeb, maneb and metiram), the thyroid was identified as the target organ (table 3). With the exception of one study using 10,000 ppm mancozeb and 1000 ppm ETU, no effects were reported on the liver for EBDCs. This was not an unexpected result considering the dietary concentrations of EBDCs used in the other rodent studies (up to 1000 ppm). The thyroid effects in the EBDC studies are qualitatively comparable to those for ETU. In studies in which a direct comparison could be made, the dose of mancozeb required was ten times higher than the dose of ETU to produce an equivalent effect on the thyroid.

1.5. Chronic studies - EBDCs

In the chronic studies (22-25) the thyroid was again identified as the target organ (table 4). No increased tumour incidence was observed in any of the long-term studies with EBDCs.
From both subchronic and chronic toxicity studies of EBDCs an overall NOAEL of 100 ppm in rats can be chosen equivalent to 5 mg/kg b.w./day. NOAELs of the other species (monkeys, dogs, mice) are of the same order of magnitude within the limitations of data available.
For mancozeb and zineb new chronic studies are in progress.

1.6. Mutagenicity of ETU

Ethylene thiourea has been tested extensively for genotoxicity in a variety of in-vitro and in-vivo systems (26-28). With a few exceptions the results are negative (table 5). In a few studies ETU induced aneuploidy and mutation in yeast and occasionally mutations in bacteria have been observed. No mutagenicity was demonstrated in insects and in in-vitro or in-vivo mammalian systems. ETU is considered not to present a mutagenic risk to mammals.

1.7. Mutagenicity of EBDC's (mancozeb, maneb, metiram and zineb)

In most of the test systems for genotoxicity the four ethylene bisdithiocarbamates gave negative results (table 6). However, there are indications that the compounds cause in-vitro chromosomal aberrations. This is reflected in the positive sister chromatid exchange assays with mancozeb (41) and metiram (43), [equivocal with maneb (42)] and the positive in-vitro cytogenetic assay with zineb (40). This chromosomal damaging effect is not found in the in-vivo cytogenetic assays. Chromosome aberrations are not related to the formation of ETU since all tests of ETU in this respect are negative.

In an operator monitoring study peripheral blood lymphocytes of workers in Czechoslovakia exposed to the fungicide Novozir Mn 80 containing mancozeb were analysed. There was a slight increase of chromosome aberrations in the group exposed to mancozeb in comparison with the control group [Jablonicka 1986 - (60)]. However, the study was severely compromised because of uneven distribution of smokers or non-smokers since smoking is known to increase chromosome aberrations.
Moreover, in-vivo tests of chromosome aberrations with EBDCs in experimental animals were all negative.

1.8. Reproductive toxicity of ETU

Reproductive toxicity studies have demonstrated the teratogenic potential of ETU in rats. Relevant findings are summarized in Table 7. Other species have not been adequately investigated to draw firm conclusions. In rats, abnormalities induced by ETU included exencephaly, cleft palate and hydrocephaly at doses in excess of 10-20 mg/kg bw/day. Investigative studies involving co-administration of ETU and thyroid hormones confirmed that the teratogenic potential of ETU could be reduced. Because of the doses used and the known relationship between fetal development and hormonal balance, it is considered acceptable to conclude that the teratogenic potential of ETU is associated with its effects on thyroid hormone balance.

1.9. Reproductive toxicity of EBDC's

Reproductive toxicity investigations carried out with EBDC's are summarized in Table 8. Teratogenicity and associated effects were clearly seen in rat studies, but not in rabbits. These findings are consistent with the proposal that the teratogenic potential is associated with ETU and its effects on thyroid hormones, where the rat is the most susceptible species. The possibility of metal chelation being associated with dithiocarbamates having an effect on the outcome of pregnancy cannot be discounted. However there is no indication of toxicological effects caused by metal chelation in any other EBDC studies and therefore this seems to be an unlikely explanation of effects seen in teratology studies. Teratogenicity was generally seen at doses around 500 mg/kg/day or greater. NOAELs were approximately 200 mg/kg/day for teratogenicity and 50 mg/kg/day for maternal toxicity and fetotoxicity.

Toxicological overview and estimation of ADI

For translation of no effect levels determined in toxicity studies in animals into acceptable daily intakes it is necessary to consider the safety factor to be used. In conventional considerations, a safety factor of 100 is used to allow for extrapolation from animals to man and for variations within the human population. In the case of EBDC and ETU a special consideration of the required safety factor is needed, in view of the unusual nature of the toxicological effects.

There are two main reasons for special consideration of safety factors with these materials. The first relates to the mechanism of action which is associated with hormonal imbalance, the second relates to the use of the rat as a model for human exposure.

When a mechanism of toxicity is associated with hormonal imbalance it is very important to take into account whether continued, persistent hormonal imbalance is required to induce toxicological changes. In the case of ETU and EBDC's, occasional exposure which may alter thyroid hormone levels would not lead to overt toxicological change in the thyroid.

In using the rat as a model for humans, it is assumed that physiological processes in the two species are similar. There are a number of points which indicate that in the case of thyroid hormone control, rat and man are very dissimilar. Thyroid hormones are transported in blood as plasma protein complexes. In humans the main carrier protein is thyroxine binding globulin. This carrier protein is not found in rodents, cats or rabbits. As a consequence the half life of T_4 is much longer in man than in rats and serum TSH levels are much lower. In addition the response of the thyroid to prolonged stimulation is different in rat and man. In rats, thyroid tumours are seen, in man, prolonged stimulation, as is seen in regions of endemic iodine deficiency, leads to goitres, but does not lead to thyroid tumours. In view of these factors it could be argued that a safety factor of 10 rather than 100 is justified.

A safety factor of 100 is therefore a prudent approach in this situation. This prudence is justified in order to make extra allowance for regional variations in thyroid function in man. it is known that in certain geographical regions, communities exist in which thyroid function is depressed in comparison with the majority of the population at large.

It is difficult to select a crucial no effect level from the range of studies conducted with ETU. Many of the experiments were of inadequate design and poorly conducted. However, from the studies conducted by Freudenthal (1977) and Graham (1975) it would seem appropriate to select a NOAEL of 25 ppm equivalent to 1.25 mg/kg bw/day. This would therefore give an ADI of 0.01 mg/kg bw. For EBDCs themselves the appropriate no effect level is 100 ppm equivalent to 5 mg/kg bw/day, giving an ADI of 0.05 mg/kg bw.

Taking into account the no effect level of 15 mg/kg bw/day ETU based on an increased incidence of liver tumours in the 2 year mouse study, a large margin of safety is present compared to the proposed ADI for ETU. The same is true when the teratological effects of EBDC's are considered, for which no effect levels of around 200 mg/kg bw/day for teratogenicity and 50 mg/kg bw/day for maternal toxicity were established in comparison with the ADI of 0.05 mg/kg bw.

1. TOXICOLOGICAL ASPECTS

1.1. Metabolism

Data on the metabolism of propineb and propylenethiourea (PTU) are available only in the rat.

In male Sprague-Dawley rats, dosed orally with 5 or 50 mg 14C-propineb/kg bw, 60-70% of the radioactivity is absorbed in 48 hours. Around 50% of the activity is excreted in the urine and about 40% via the feces within this period. Exhalation during the first 24 hours after administration amounted to 7% and excretion in the bile to 3% of the administered dose (76, 77, 78).

Peak levels in blood and most organs are reached 3-5 hours after administration, whereas in the thyroid the maximum concentration is attained after 24 hours. Within 4 days the total elimination exceeds 99%. The half live of elimination from the tissues (except the thyroid) is first rapid than slower, varying between 5 and 20 days. Four days post dosing (50 mg/kg) the concentration of radioactive material in the thyroid is about 100 times and in the kidney and pituitary 3-4 times higher than the average concentration for all other organs except the gastrointestinal tract. After 16 days the relative concentration factor for the thyroid is about 30 (79, 80).

The main metabolites found in the urine of the dosed rats were propylene diamine (accounting, on a molar basis, for 12-15% of the renally excreted activity), and PTU and propylene urea (PU) [accounting together for 40-45%]. A minor metabolite is probably 4-methylimidazoline (less than 5%). No compounds could be considered as potential intermediates for complete degradation to CO2 (76, 77).

PTU is regarded as the main metabolite responsible for the goitrogenic/tumorigenic actions of propineb, but the available data on toxicokinetics are even more sparse than those of propineb. The data show remarkable differences to those obtained with propineb.

Following oral administration of PTU (0.5, 5.0 or 50.0 mg/kg bw) rats absorbed approximately 95% via the digestive tract. A total of 85 to 92% of the dose applied was eliminated in the urine, 7-14% in the feces and less than 0.2% by exhalation within 48 hours. Excretion in the bile amounted to 22% within 24 hours after intraduodenal application, indicating enterohepatic circulation in rats (81).

Peak concentration in the thyroid is reached after 8 hours, being almost 6 times higher than after an equivalent dose of propineb. After 1-10 days post dosing the concentration of radioactivity in the thyroid after equivalent doses of PTU and propineb ranges between 3.3 and 5 times higher for PTU than for propineb (78). Mean tissue concentrations (except of the gastrointestinal tract) decreased by a factor of 50 in the course of the first 2 days, and thereafter much more slowly, but in the thyroid the radioactivity dropped only to about one-seventh between 8 hours and 10 days post application. Since there was a strong dose-proportionality in the dose range tested it was concluded that residues in the tissues at still lower doses will be proportionally lower (81).

No data were available on the metabolic fate of PTU.

1.2. Subchronic studies - PTU

There is only one relevant subchronic toxicity study with PTU. In this study rats received daily oral doses of 50 mg/kg bw for 21 days of propineb, PTU, ETU, zineb or methylthiouracil. PTU was revealed to be as effective as methylthiouracil in its goitrogenic activity and somewhat stronger than ETU (82).

The subchronic study, summarised in references 77 and 79 deals with the comparison of ETU to propylthiouracil, shortened in the original paper misleadingly as "PTU" (83). The same is true with respect to the paper of Cooper (84), summarised in a comparative toxicological profile of ETU, submitted by Bayer AG (85).

1.3. Chronic studies - PTU and PU

Chronic toxicity studies have been conducted with PTU in rats and mice (Table 9).

A 2-year study on Wistar rats was performed with dietary levels of 0 (Control), 1, 10, 100 and 1000 ppm (equivalent to 0, 0.05, 0.5, 5.0 and 50.0 mg/kg bw/day). Administration of 1000 ppm caused high and early (up to the 65th week) mortality accompanied by severe clinical signs, reduced weight gain, impaired hematopoiesis, damage of liver and kidney and extremely high cholesterol levels. The thyroid weight was about 6-fold enhanced and the level of protein bound iodine (PBI) in plasma was 2-3 times higher than in controls. In addition to nodular hyperplasias in the thyroid, adenomas and cystadenomas developed, but without signs of malignant transformation. At 100 ppm there was still an impairment of growth and a raised cholesterol level. The increase of the thyroid weight was accompanied by an increased incidence of nodular hyperplasia in males and females. In females the nodules were accompanied by large follicles, colloid cysts and vacuolated epithelial cells. Administration of 1 or 10 ppm did not reveal any significant test compound induced thyroid alterations. The NOAEL was estimated to be 10 ppm, equivalent to 0.5 mg/kg bw/day (86, 87).

CFI mice were fed with dietary concentrations of 0 (Control), 1, 10, 100 or 1000 ppm (equivalent to 0, 0.15, 1.5, 15.0 and 150 mg/kg bw/day) for 2 years. The highest dosage was applied alternating every other week for 7 days and a satellite group was autopsied after 12 months. Appearance, behaviour, feed intake and mortality remaind unaffected in the male and female mice of all dose groups. Administration of 1000 ppm resulted in reduced body weight development, increased liver weight, elevated activities of alkaline phosphatase and AST in plasma, increased cholesterol level, and enlarged thyroids with increased number and size of follicle cells coupled with reduction in follicle size in males. Most findings were mentioned also at 100 ppm, except for changes of the thyroid and of plasma cholesterol. From 10 ppm the incidence of hepatocellular adenomas and carcinomas was increased, being dose-related for carcinomas in males and females starting at the lowest dose and not clearly dose-related for adenomas. The tumour incidence is summarised below:

Table 7: Reproductive toxicity studies of ETU

No	Characteristics of the studies (a,b,c)[1]	Lowest dose (mg/kg bw/day) with an adverse effect on dam and/or fetus			Remarks
		maternal toxicity	feto-toxicity	terato-genicity	
1.	Rabbits a. Khera 1973(62) b. 10 - 80	-	80	80	questionable results. Small group size and little detail publication.
2.	Rats 2.1 a. Khera - 1973(62)				
(a)	b. 5-40 c. premating -d 15	> 40	>5	>20	
(b)	b. 5-80 c. 6-15 d	> 80	>5	>10	
(c)	b. 5-40	> 40	-	>10	
	2.2				
3.	a. Ruddick 1975(63) b. 40 - 480 c. 6 - 21 d (one day)	-		+	The most sensitive days of gestation were 12-15th. Details are given at 240 mg/kg Malformations dose related
4.	a. Lu 1978(64) b. 40 (+/- T4) c. 7-15 d.			+	Co-administration of T4 reduced but not totally abolished teratogenic potential or ETU.
5.	a. Emmerling 1977(65) b. 20-40 (+/- T3, T4) c. 7-20 d.			+	T3 or T4 co-administration with ETU, markedly reduced malformations.
	Hamster				
6.	a. Khera 1983(66) b. 600 - 2400 c. 11 d.	-	>1200	>1200	

1) a: reference, b: range of doses used in mg/kg/day, c: period (in days) of treatment during the gestation.

Table 8: Reproductive toxicity studies of EBDC's

No	Characteristics of the studies (a,b,c)*	Lowest dose (mg/kg/day) with an adverse effect on dam and/or on fetus			Remarks
		maternal toxicity	feto-toxicity	terato-genicity	
	<u>mancozeb-rat</u>				
1.	a. Larsson, 1976(67) b. 380 - 1320 c. 11 d			1320	
2.	a. Stevens, 1980(68) b. 2-512 c. 6-15 d (+ETU 50 mg/kg)	>128	128/512	128/512	The fetotoxicity and teratogenicity observed at the dose 128 mg/kg bw/day was questionable
	<u>mancozeb-rabbits</u>				
3.	a. Solomon, 1987a(69) b. 50-110 c. 7-19 d.	>100	-	>100	Poor survival precluded investigations at doses higher than 100 mg/kg bw/day.
4.	a. Solomon, 1987b(70) b. 10-80 c. 7-19d	80	-	-	
	<u>maneb - rat</u>				
5.	a. Hofmann, 1976(71) b. 20-500 c. 6-15 d	500	500	500	
6.	a. Merkle, 1983(72) b. 5-80 c. 6-18 d.	80	-	-	
	<u>metiram-rat</u>				
7.	a. Palmer, 1979b(73) b. 40-160 c. 6-15 d	>80	160	-	
	<u>metiram-rabbit</u>				
8.	a. Hellwing, 1988(74) b. 10-120 c. 7-19 d.	40/120	-	-	
	<u>zineb-rat</u>				
	a. Short, 1980(75) b. 200-2000 c. 6-19	2000	2000	2000	

a: reference, b: range of doses used in mg/kg bw/day, c: period (in days) or day of treatment during gestation.

Table 9: Chronic feeding toxicity studies of PTU and PU

Species	Dose*	Duration	Effects	NOAEL (mg/kg bw/day)	Ref
Rat	0,0.05,0.5, 5,50 (PTU)	2 years	mortality thyroid weight and tumours weight gain liver damage	0.5	86,87
Mouse	0,0.15,1.5, 15,150# (PTU)	2 years	weight gain liver damage liver tumours thyroid weight	0.15	88
Mouse	0,7,70,300 (PU)	2 years	liver damage liver tumours no thyroid effects	7	89

*: ppm in diet converted to mg/kg bw/day
#: alternate weeks dosing

Refs: 86: Weber and Patschke, 1979
87: Bomhard and Loser, 1980
88: Bomhard and Loser, 1981
89: Krotlinger and Loser, 1981

Table 10: Subchronic feeding toxicity studies of propineb

Species	Dose*	Duration	Effects	NOAEL (mg/kg bw/day)	Ref
Rat	0,100,400	12; 8 days	thyroid weights (reversible)	-	90
Rat	50	21 days	thyroid weight (reversible)	-	82
Rat	0,0.25,0.5, 1.25,2.5,5.0	13 weeks	no thyroid effects, enzyme effects	1.25	91,92
Rat	0,1,2.5,10, 25	13 weeks	liver weight, Glob/Alb	-	93
Rat (male)	0,0.1,0.5, 2.5,12.5	62 days	iodine uptake T3 + T4 thyroid weight	0.5	94,95
Dog	0,1.25,12.5 50	13 weeks	disturbed lipid metabolism urea N	-	96
Dog	0,2.5,10,40	4 months	liver weight spleen weight	2.5	97
Dog	0,2.5,7.5, 25,75	2 years	impaired hepatic function	25	98

*: ppm in diet converted to mg/kg bw/day

Refs:
90: Loser and Lorke, 1967
82: Kimmerle, 1972
91: Loser, 1969
92: Loser, 1968
93: Sterner, et al., 1977
94: Weber and Dressler, 1980
95: Krotlinger, et al., 1980
96: Sterner, et al., 1977
97: Loser, 1967
98: Loser, 1973

Table 11: Chronic feeding toxicity studies of propineb

Species	Dose*	Duration	Effects	NOAEL (mg/kg bw/day)	Ref
Rat	0,0.25,0.5, 1.25,2.5,5	2 years	slight hepatic effects no thyroid effects	2.5	99
Rat	0,0.05,0.5 5,50,100, 400	2 years	mortality thyroid weight thyroid tumours slight hepatic effects	0.5	100
Mouse	0,7,29,114	2 years	liver tumours thyroid weight	29	101

*: ppm in diet converted to mg/kg bw/day

Refs: 99: Loser, 1974(a)
100: Loser, 1974(b)
101: Brune and Deutsch-Wenzel, 1980

Table 12: Mutagenicity studies of PTU/PU

Test	Concentration/ Dose	Results	Ref
PTU			
Reverse mutation Salmonella Typhimurium* TA98,TA100,TA1535,TA1537	20-12500 µg/plate	Negative	102
Reverse mutation Salmonella Typhimurium* TA98,TA100,TA1535,TA1537	20-12500 µg/plate	Negative	103
E Coli Pol A*	62-1000 µg/plate	Negative	105
DNA investigations in CF1 mice	100 mg/kg bw	Negative	106
PU			
Reverse mutation Salmonella Typhimurium* TA98,TA100,TA1535,TA1537	20-12500 µg/plate	Negative	104

*: with and without activation

Refs: 102: Herbold, 1980(b)
103: Herbold, 1981(a)
104: Herbold, 1980(c)
105: Herbold, 1981(b)
106: Altmann, et al, 1981

Table 13: Mutagenicity studies of propineb

Test	Concentration/ Dose	Results	Ref
Reverse mutation Salmonella Typhimurium* TA98,TA100,TA1535,TA1537	20-12500 µg/plate	Negative	107
Reverse mutation E Coli WP2 hcr-* Salmonella Typhimurium* TA98,TA100,TA1535,TA1537	0.09-864 µg/plate	Negative	108
Rec assay B Subtilis H17 rec+ M45 rec-	0.9-864 µg/plate	Negative	108
CHO-HGPRT assay*	0.11-60 µg/ml	Negative	110
UDS primary rat hepatocytes	5-30 µg/ml	Negative	109
Micronucleus test NMRI mice	2x1000 mg/kg bw or 2x2000 mg/kg bw	Negative	111
Dominant lethal test NMRI mice	500 mg/kg bw	Negative	112

*: with and without activation

Refs: 107: Herbold, 1980(a)
108: Hatano Institute, 1978
109: Clfone, 1987
110: Lehn, 1988
111: Herbold, 1982
112: Machemer, 1974(a)

Table 14: Reproductive toxicity studies of PTU

Species	Dose*	Duration (Days pc)	Maternotox at dose	Teratogenic at dose	NOAEL	Ref
Rat	0,11.25,22.5, 45.0,90.0	6-15	90	45+90	22.5	113
Rat	0,20,50,75, 100,200	13	?	50+>	20	114
Rat	240	12 or 13	?	240(100%)	-	115

*: mg/kg bw/day

Refs: 113: Vicari, et al., 1985
114: Bleyel and Lewerenz, 1978
115: Ruddick, et al.,1976

Table 15: Reproductive toxicity studies of propineb

Species	Dose*	Duration (Days pc)	Maternotox at dose	Teratogenic/ embryotoxic at dose	NOAEL	Ref
Rat	0,3,10,30, 100	6-15	100	100	30	116
Rat	0,25,50,100, 200	6-15	100+200	100+200	50	113
Rat	0,400,760, 2300	11	400+>	2300	-	117
Rabbit	0,10,30,100	6-18	30+100 (impl. losses)	-	10	118

*: mg/kg bw/day

Refs: 113: Vicari, et al, 1985
116: Machemer, 1974
117: Larsson, et al, 1976
118: Becker, et al, 1988

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		Tumour incidence in % at dietary level (ppm):				
		0	1	10	100	1000
Carcinomas	m	0	13	29	31	21
	f	2	2	5	26	38
Adenomas	m	0	11	10	7	26
	f	0	10	13	32	18

It was noted that the hepatic tumour incidence in control animals was unusually low in this study and that the incidence of tumours at the lowest dosage lies inside the historical control range for these neoplastic lesions in CFI mice. Based upon comparison with historical control data, the NOAEL was estimated to be 1 ppm, equivalent to 0.15 mg/kg bw/day (88).

A 2 year feeding study in mice with propylene urea (PU) at dietary levels of 0 (Control), 50, 500 and 2500 ppm (equivalent to 0, 7, 70 and 300 mg/kg bw/day) revealed, at 2500 ppm, ocular opacities, impaired kidney function and hematopoiesis, liver damage, hyperplasia of the adrenal cortex, and an increased incidence of liver cell adenomas and carcinomas. At 500 ppm centrilobular hyperplasia in the liver was noted. Administration of 50 ppm, equivalent to 7 mg/kg bw/day, was apparently without any detectable effect (89).

In summary an NOAEL for PTU of 0.5 mg/kg bw/day for rats seems justified and an NOAEL of 0.15 mg/kg bw/day may be derived in mice.

1.4. Subchronic studies - propineb

In a number of subchronic toxicity studies with propineb (82, 90-98), the thyroid and liver were identified as target organs (Table 10). In general, thyroid and liver effects were seen but thyroid effects were not seen in dogs. The thyroid changes seen with propineb are qualitatively similar to those observed for PTU and those previously discussed for ETU and EBDC's. Although direct comparisons are difficult, the data suggest that propineb and PTU are more potent inducers of thyroid hyperplasia than EBDC's and ETU.

Summarising the subchronic studies, an overall NOAEL may be derived in rats of 1.25 mg/kg bw/day and in dogs of 2.5 mg/kg bw/day.

1.5. Chronic studies - propineb

Two 2-year Wistar rat feeding studies were reported in 1974 and a long term mouse study has also been conducted. The results are summarised in Table 11.

In the first rat study, performed with dietary levels of 0 (Control), 5, 10, 25, 50 and 100 ppm (equivalent to 0.25, 0.5, 1.25, 2.5 and 5 mg/kg bw/day), there was no effect on food consumption, growth rate and mortality at any dose level. Dose-related histopathological changes were not observed in any organ, including the thyroid. Treatment with 100 ppm occasionally caused slightly increased ALAT* and ASAT* activities; 50 ppm provided no indication of liver damage. Nature, location and frequency of tumours gave no indications for any carcinogenic effects of the test compound. An NOAEL of 50 ppm, equivalent to 2.5 mg/kg bw/day was derived (99).

The second rat study used dietary levels of 0 (Control), 1, 10, 100, 1000, 2000 and 8000 ppm (equivalent to 0.05, 0.5, 5.0, 50.0, 100 and 400 mg/kg bw/day). Premature death occurred at 2000 and 8000 ppm in all females and at 8000 ppm in all males. Signs of myasthenia and histological alterations of skeletal muscles were observed at 1000 ppm and above. Absolute and relative liver weights in males were increased at 100 ppm and above but histological alterations were not observed. Significant enlargement of the thyroids was observed in males at 100 ppm and above and in females at 1000 ppm and above. Dose related hyperplasias of the thyroid as well as thyroid adenomas increasingly occurred starting at 1000 ppm. An NOAEL of 10 ppm, equivalent to 0.5 mg/kg bw/day was derived (100).

A 104 week carcinogenicity study was performed in NMRI mice with dietary levels of 50, 200 and 800 ppm (equivalent to 7, 29 and 114 mg/kg bw/day). Neither clinical signs nor clinical chemistry or urine analysis data were affected at any dose level. There was some indication of increased thyroid weight in females and an increase of hepatocellular adenomas was observed in males at the highest dose group (controls 8%; 50 ppm 6%; 200 ppm 4%; 800 ppm 18%). Inter-group differences in the incidence of lung adenomas were observed in females, varying from 19% (0 ppm) to 27% (50 ppm), 30% (200 ppm) and 50%

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*: ALAT = Alanine Aminotransferase, formerly known as GPT

*: ASAT = Aspartate Aminotransferase, formerly known as GOT

in females, varying from 19% (0 ppm) to 27% (50 ppm), 30% (200 ppm) and 50% (800 ppm). These incidences were all within the historical control range and may have been affected by the higher survival rates at higher concentrations of the test substance, therefore, the NOAEL is 200 ppm, equivalent to 29 mg/kg bw/day (101).

In summary, for propineb, overall NOAEL's of 2.5 mg/kg bw/day for rats and 29 mg/kg bw/day for mice may be derived.

1.6. Mutagenicity - PTU and PU

Mutagenicity tests conducted with PTU and PU are summarised in Table 12.

The activated and non-activated assay on different strains of Salmonella typh. as well as an E.coli pol A1 Test (102, 103, 105) revealed no indications for point mutations with PTU. Investigation of the interaction of PTU with DNA in mice (100 mg PTU/kg bw oral) showed only an enhanced semiconservative DNA synthesis. Binding of PTU to mice liver DNA could not be detected and there was no indications of unscheduled DNA synthesis or strand breaks (106). No information on possible effects on chromosome aberrations in vitro is available, nor on gene mutation in mammalian cells, and it is not possible to draw any firm conclusions regarding the mutagenic potential of PTU.

A Salmonella/microsome test in 4 strains with propylene urea in concentrations up to 12500 µg/plate revealed no evidence for mutagenic activity (104).

1.7. Mutagenicity - propineb

Mutagenicity assays conducted with propineb are summarised in Table 13.

All in vitro and in vivo assays performed gave no indications for mutagenic actions of propineb. Even cytotoxic concentrations did not result in positive findings (107-111). A dominant lethal assay showed impaired fertility of male mice, dosed orally with 500 mg/kg bw/day but revealed no indications for mutations in germ cells (112).

Although, as with PTU, studies on chromosome aberrations in vitro were not carried out, it may be concluded that propineb did not demonstrate any mutagenic activity.

1.8. Reproductive toxicity - PTU

The teratogenic potential of PTU has been investigated in the rat and the study results are summarised in Table 14.

According to Vicari, et al. (113) PTU showed a clear teratogenic activity in a conventional rat teratology study at doses which did not show maternal toxicity. Earlier studies (114, 115) showed that PTU has teratogenic potential in rats following one or two doses on days 12 or 13 of gestation.

In summary the NOAEL for teratogenic effects of PTU derived from these few studies may be approximately 20 mg/kg bw and effects occur at non or marginal maternotoxic dosages.

1.9. Reproductive toxicity - propineb

The teratogenic potential of propineb has been investigated in the rat and rabbit and general effects on reproductive function have been investigated in a multigeneration study in rats. The results of these studies are summarised in Table 15.

The rat teratology study revealed visceral and skeletal abnormalities at high doses accompanied by maternal toxicity (113, 116, 117), while in rabbits an increased implantation loss was observed, but no fetal abnormalities (118).

A 3-generation study in rats with dietary levels of 0 (Control), 20, 60, 200 and 600 ppm (equivalent to 1, 3, 10 and 30 mg/kg bw/day) resulted in impaired well-being of parental animals at 200 and 600 ppm, along with reduced pregnancy rate and reduced number of live offspring. An NOAEL of 60 ppm (equivalent to 3 mg/kg bw/day) may be derived (119).

In summary, an overall NOAEL of 50 mg/kg bw/day can be set in rats for teratogenic effects of propineb, being at the margin of maternal toxicity. Rabbits show a much lower sensitivity, no evidence of teratogenicity was seen at 100 mg/kg bw/day. The 3-generation study in rats resulted in a NOAEL of 3 mg/kg bw/day.

1.10. Observations in humans on propineb

The health of workers exposed to propineb was reported not to be impaired. There were also no indications of malfunctions of the thyroid or irritant or sensitizing actions to the skin (120-122).

Toxicological overview and estimation of ADI

The effects of propineb seem chiefly to be due to its main metabolite propylenethiourea (PTU). Both propineb and PTU very closely resemble, with respect to their toxicological profile as well as to their chemical structure, the ethylenebisdithiocarbamates (EBDC's) and their common main metabolite ethylenethiourea (ETU). Propineb, PTU, EBDC's and ETU affect the function of the thyroid, causing a compensatory enlargement of the gland by TSH-stimulation. In addition, liver function becomes altered, resulting in disturbances of lipid metabolism, e.g. enhanced cholesterol levels, and increased liver weight. Both actions may be connected to each other by the thyroid-liver hormonal axis. At higher dosages overstimulation of the thyroid by TSH leads to hyperplasia, adenomas and with PTU and ETU also to carcinomas of this gland in rats. In mice an increased incidence of hepatomas was observed with ETU and PTU (ETU, Ref. 15). Mutagenicity studies revealed no indications neither with EBDC's or ETU nor with propineb or PTU, although with PTU the data are rather limited. Teratology studies also show strong similarities between EBDC's and ETU on the one and propineb and PTU on the other hand, both latter compounds being effective slightly below or at the border of maternal toxicity.

In view of the intimate relationships between EBDC's/ETU and propineb/PTU it seems justifiable to treat these groups of compounds identically. If thresholds are assumed for the tumorigenic effects of EBDC's/ETU this should be accepted also for propineb/PTU. With regard to estimation of an ADI the crucial no adverse effect level for propineb is considered to be 1.25 mg/kg bw/day. Although lower no effect levels were derived for individual studies a dose of 1.25 mg/kg bw/day is the highest no adverse effect level which is still lower than the actual adverse effect levels from all the studies. For PTU, a no adverse effect level of 0.15 mg/kg bw/day can be chosen from the mouse study, even though the effects seen in mice might be considered to be of no relevance to man. With respect to the teratogenic actions, in rats for

propineb a NOAEL of 50 mg/kg bw/day and for PTU of approximately 20 mg/kg bw/day, given during the sensitive stages of gestation, can be deduced. These NOAEL's are about 50 times higher than the NOAEL's derived from the subchronic and chronic studies with PTU and propineb.

Considering the generally weak database for propineb/PTU in comparison with EBDC's/ETU, (in particular the poor metabolism data, the lack of information on the conversion of propineb to PTU and the high degree of uncertainty in selecting the no effect level for PTU) it seems prudent to apply at least a safety factor of 200 to the NOAEL's of propineb and PTU, to derive an ADI. The ADI is thus estimated at 0.006 mg/kg bw for propineb and 0.0008 mg/kg bw for PTU. Those values are considerably lower than the ADI's derived for EBDC's of 0.05 mg/kg bw and for ETU of 0.01 mg/kg bw.

The toxicological potencies of propineb/PTU and EBDC's/ETU seem, based on currently available data, to be sufficiently different to require separate ADI's. However, the analytical method commonly used to determine residues of these materials in food (based on CS2 generation) cannot distinguish between residues of propineb and EBDC's. It is therefore strongly recommended that further work be undertaken to develop and validate a new analytical method to specifically detect residues of propineb/PTU.

As long as such a specific method is not available, consideration should be given to:

- Use of the ADI's for propineb/PTU for all pesticides giving rise to CS2 on analysis,
- Withdrawal of use of propineb in those cases giving rise to residues in edible crops,
- Total withdrawal of approval for use of propineb.

Table 1: Subchronic feeding toxicity studies of ETU

N°	Characteristics of the studies (a, b, c)*	Lowest dose (in ppm) with an adverse effect on body weight and on thyroid					NOAEL (in ppm)
		Body weight gain (decrease)	Thyroid weight (increase)	Thyroid hyperplasia	Thyroid tumours "adenomas"	Thyroid hormones (T_3/T_4,THS)	
A.	RATS						
1.	a. (3) b. 50 - 750 c. 13 w	100	50	100	500	--	none
2.	a. (4) b. 60 - 750 c. 13 w	500	--	250	250	--	60 (based on thyroid weight)
3.	a. (5) b. 10 - 1.000 c. 12 w	--	159	--	--	--	63 (based on thyroid weight)
4.	a. (6) b. 1 - 625 c. 13 w	625	--	125	--	125	25 (based on T_4 levels)
5.	a. (7) b. 0,625 - 25 c. 8 w	-(>25)	-(>25)	-(>25)	--	5	1.25(based on increased protein bound iodine) (question. findings)
6.	a. (8) b. 75 - 150 c. 7 w	75	75	--	--	150	none
7.	a. (9) b. 125 - 625 c. 12 w	--	125	125	--	125	none
B.	MOUSE						
1.	a. (10) b. 125 - 2.000 c. 13 w	>2.000	--	500	--	--	250 (based on thyroid hyperplasia)
2.	a. (11) b. 1 - 1.000 c. 13 w	>1.000	1.000	100	--	--	10 (based on thyroid hyperplasia)
C.	MONKEY						
1.	a. (12) b. 2 - 250 c. 22 - 24 w	>250	--	50	--	50	10
2.	a. (12) b. 50 - 450 c. 26 w	>450	50	50	--	150	none

* a: reference number, b: range of doses used in ppm, c: duration of the experiment in weeks.

(3): GRAHAM 1972, (4): PETERS 1980, (5): HART 1973, (6): FREUDENTHAL et al. 1977, (7): LEUSCHNER 1977, (8): ARNOLD 1983, (9): LEBER 1977, (10): PETERS 1980, (11): O'HARA 1985, (12): LEBER 1978.

Table 2: Chronic feeding (2 years) studies for ETU in rats

N°	Characteristics of the studies (a, b, c,)*	Lowest dose (in ppm) with an adverse effect on body weight and on thyroid					NOAEL (in mg/kg diet)
		Body weight gain (decrease)	Thyroid weight (increase)	Thyroid hyperplasia	Thyroid tumours "adenomas"	Thyroid hormones (T_3/T_4, THS)	
1.	a. rat - (13) b. 5 - 500 c. 104 w	500	125	?	250	--	25
2.	a. rat - (14) b. 5 - 200 c. 104 w	17	60	--	60	--	Not sufficiently well reported to set a NOAEL

* a: animal species and reference number, b: range of doses used in ppm, c: duration of the experiment in weeks.
? : Inadequate data to establish a NOEL
(13): GRAHAM et al., 1975.
(14): GAK et al., 1976.

NTP studies are not included as they have not been fully reported (see pages 2 and 3) at the time of writing.

Table 3: Subchronic feeding toxicity studies of EBDCs

N°	Characteristics of the studies (a, b, c, d)*	Lowest dose (in ppm) with an adverse effect on body weight and on thyroid					NOAEL (in ppm)
		Body weight gain (decrease)	Thyroid weight (increase)	Thyroid hyperplasia	Thyroid tumours "adenomas"	Thyroid hormones (T_3/T_4,THS)	
1.	a. mancozeb b. rat - (16) c. 30 - 1.000 d. 13 w	1.000	--	1.000	--	250	125
2.	a. metiram b. rat - (17) c. 50 - 900 d. 13 w	900	--	900	--	300	100
3.	a. mancozeb b. mouse - (11) c. 10 - 10.000 d. 13 w	10.000	10.000	1.000	--	--	100
4.	a. mancozeb b. dog - (18) c. 10 - 5.000 d. 13 w	1.000	5.000	5.000	--	5.000	100
5.	a. mancozeb b. dog - (19) c. 25 - 1.000 d. 104 w	1.000	--	1.000	--	--	100
6.	a. maneb b. monkey - (20) c. 100 - 3.000 d. 26 w	3.000	300	3.000	--	300	100
7.	a. metiram b. monkey - (21) c. 5 - 75 d. 26 w	>75	75	15	--	15	5**

* a: product; b: animal species and reference number, c: range of doses used in ppm, d: duration of the experiment in weeks; **: expressed in mg/kg b.w./day.

(16): GOLDMAN 1986.
(17): HUNTER 1977.
(11): O'HARA 1985.
(18): COX 1986.
(19): BORZELLECA et al. 1965a
(20): LEUSCHNER 1977.
(21): SORTWELL 1979.

Table 4: Chronic feeding (2 years) studies of EBDCs

N°	Characteristics of the studies (a, b, c, d)*	Lowest dose (in ppm) with an adverse effect on body weight and on thyroid					NOAEL (in ppm)
		Body weight gain (decrease)	Thyroid weight (increase)	Thyroid hyperplasia	Thyroid tumours "adenomas"	Thyroid hormones (T_3/T_4, THS)	
1.	a. mancozeb b. rat - (22) c. 25 - 1.000 d. 90 w	>1.000	1.000	1.000	--	--	**100**
2.	a. maneb b. rat - (23) c. 30 - 1.000 d. 132 w	1.000	1.000	>1.000	--	1.000	300
3.	a. metiram b. rat - (24) c. 5 - 320 d. 111 - 119 w	>320	>320	>320	--	320	**80**
4.	a. metiram b. mouse - (25) c. 100 - 1.000 d. 88 - 96 w	1.000	>1.000	>1.000	--	--	**300**

* a: product; b: animal species and reference number, c: range of doses used in ppm, d: duration of the experiment in weeks.

(22): BORZELLECA et al. 1965b.
(23): LEUSCHNER 1979.
(24): HUNTER 1981.
(25): HUNTER 1979.

Table 5: Mutagenicity tests of ETU[1]

TESTS	Total No. of tests	RESULTS		
		positive +	equivocal (+)	negative -
In vitro				
1. Procaryotes				
-gene mutation { -activation	31	4	2	25
-gene mutation { +activation	30	8	4	18
2. Yeasts				
-gene conversion { -activation	9	4	1	4
aneuploidy (mutation { +activation	7	1	-	6
3. Mammalian cells				
- gene mutation	3	-	-	3
- chromosomal aberration	3	-	-	3
- sister chromatid exchange	3	-	-	3
- DNA repair	2	-	-	2
In vivo				
1. Host-mediated assay	3	1[2]	-	2
2. Drosophila sex linked recessive	1	-	-	1
3. Micronucleus test	5	-	-	5
4. Chromosomal aberration	2	-	-	2
5. Dominant lethal test	3	-	-	3

(1) Based mainly on review by IARC (1987) (26) with additional data from Teramato 1977 (27) and McGregor 1988 (28).

(2) found at 6000 mg/kg bw.

Table 6: Mutagenicity tests for EBDC's (combined)

TESTS	No of tests	RESULTS			REFERENCES
		positive	equivocal	negative	
In vitro					
1. Procaryotes					
-gene mutation { -activation	7			7	29, 30, 31, 32,
-gene mutation { +activation	6			6	33, 34, 35
-DNA repair	2			2	30, 35
2. Yeasts					
-gene mutation { -activation	1			1	30
-gene mutation { +activation	1			1	30
3. Mammalian cells					
-gene mutation { -activation	4		1	3 -	36, 37, 38
-gene mutation { +activation	4			4	39
-chromosomal aberration { -act.	1	1			40
-chromosomal aberration { +act.	1	1			40
-sister chromatid exch. { -act.	3	2		1	41, 42, 43
-sister chromatid exch. { +act.	3	2	1		41, 42, 43
-DNA repair	3			3	44, 45, 46
In vivo					
1. Host mediated assay	4			4	47, 48, 49, 50
2. Chromosomal aberration	4			4	51, 52, 53, 54
3. Dominant lethal assay	1			1	55
4. Cell transformation	5			5	56, 57, 58, 59, 60

29. CHISM 1984
30. SHIRASU 1979
31. THOMAS 1985
32. DESCH 1977
33. ENGELHARDT 1985
34. ENNINGA 1986a
35. SHIRASU 1976
36. FOXALL 1985
37. THOMAS 1986a
38. JACKH 1985
39. ENNINGA 1987
40. ENNINGA 1986b
41. IVETT 1985a
42. THOMAS 1986b
43. IVETT 1986
44. BYERS 1985
45. LOVEDAY 1986
46. CIFONE 1984
47. McCAROLL 1984
48. McCAROLL 1985a
49. McCAROLL 1985b
50. JAGANAATH 1984
51. SAMES 1984
52. IVETT 1985b
53. ZELLER 1981
54. IVETT 1986b
55. PALMER 1979
56. McGLYNN-KREFT 1984
57. McLEOD 1985
58. TU 1985a
59. TU 1986
60. TU 1985b

REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF IPRODIONE

(Opinion expressed by the SCP on 2 June 1992)

BACKGROUND AND TERMS OF REFERENCE

Iprodione is a protectant 3',5'-dichloroanilide fungicide with some eradicant activity used to control many diseases on a wide range of horticultural and agricultural crops. In the context of its work to establish maximum pesticide residue levels pursuant to the relevant Community legislation, the Commission invited the Scientific Committee for Pesticides to examine toxicological data relating to iprodione and to estimate its acceptable daily intake.

Since iprodione is structurally similar to vinclozolin and procymidone, both of which have been reported to possess anti-androgenic potential, the data to be reviewed concentrated on studies likely to reveal any anti-androgenic effects (subchronic experiments in dogs, including a new study specially conducted to define a no adverse effect level, and carcinogenicity and reproductive toxicology studies).

The toxicity of iprodione has previously been reviewed by the JMPR in 1977, when an ADI of 0.3 mg/kg bw was derived.

1. TOXICOLOGICAL ASPECTS

1.1. Toxicity studies in dogs

In the first study, reported in 1984, groups of 6 male and 6 female beagle dogs were fed diets containing iprodione (technical grade, 96.5%) at concentrations of 0 (Control), 100, 600 or 3600 ppm for 52 weeks. There were no clinical signs of reaction to treatment. Food and water consumption and body weight gain remained undisturbed by treatment. Physical examination and urinalysis revealed no treatment related effects. Ophthalmoscopy revealed that retinal hyperreflection was observed more frequently in treated animals than among controls and there was some evidence of a dose related trend among males receiving 600 or 3600 ppm. In treated females this finding was more persistent than among control females. Haematological changes consistent with haemolytic anaemia were seen at 3600 ppm (decreased haemoglobin, red cell count and packed cell volume and increased Heinz bodies). A slight increase in Heinz bodies was also seen at 600 ppm, during the first 18 weeks of the study only. Blood chemistry investigations revealed an increased alkaline phosphatase activity among dogs receiving 3600 ppm. At the end of the study, liver and adrenal weights at 3600 ppm were higher than control values, while prostate weights among males at 600 and 3600 ppm were lower than in control males. Histopathology revealed treatment related changes in

liver and urinary bladder at 3600 ppm (submucosal crystals within giant cells and granulomas within the submucosa in the urinary bladder and centriacinar hepatic cord atrophy) and stress related changes in the adrenals and kidneys at 600 and 3600 ppm. Microscopic examination revealed no treatment related change in the prostate to correlate with the intergroup difference in weight. These findings indicate that 100 ppm (equal to 4.2 mg/kg bw/day) was a no adverse effect level in this study (1, 2, 3).

In the second study, reported in 1991, groups of 6 male and 6 female beagle dogs were fed diets containing iprodione (technical grade, 96.1%) at concentrations of 0 (Control), 200, 300, 400 or 600 ppm for 52 weeks. There were no clinical signs of reaction to treatment. Food and water consumption and body weight gain remained undisturbed by treatment and ophthalmological examinations revealed no treatment related effects. Although there was some indication of a slight reduction in red cell parameters in treated females and in males receiving 600 ppm, all values were similar to pre-treatment values and within the range of normal biological variation for dogs of this age. There was no indication of Heinz bodies in erythrocytes from control or treated dogs at any stage. Organ weight analysis and histopathology conducted at termination revealed no indication of any reaction to treatment. These findings show that dietary administration of iprodione to beagle dogs at levels up to 600 ppm produced no indication of a toxic effect in this study (4).

1.2. Chronic toxicity/Carcinogenicity

In a rat study reported in 1978, groups of 60 male and 60 female Sprague-Dawley rats were fed diets containing 0 (Control), 125, 250 or 1000 ppm iprodione (purity unspecified) for 24 months (the high dose group received 500 ppm for the first 8 days of the study). Mortality remained unaffected, while food consumption was slightly decreased in treated groups and there was a corresponding decrease in weight gain. These intergroup differences in food intake and weight gain, although statistically significant, were small and not dosage related in degree and were considered to be of no toxicological significance. Clinical chemistry, haematology and urinalysis revealed no evidence of reaction to treatment. Organ weight data were affected by the minor intergroup disparity in body weight. However, there were no consistent changes which were considered to be of any toxicological significance. Histopathological examination revealed no treatment related effects and in particular there was no indication of any tumorigenic potential of iprodione. The no observable adverse effect level was considered to be in excess of 1000 ppm which was equal to 49 mg/kg bw/day (5).

In a mouse study reported in 1978, groups of 60 male and 60 female CF-1 mice were fed diets containing 0 (Control), 200, 500 or 1250 ppm iprodione (purity unspecified) for 18 months. There were no clinical signs of reaction to treatment and survival rates of control and treated mice were similar. Weight gain and food intake remained undisturbed by treatment and ophthalmoscopic examination revealed no indication of any reaction to treatment. Results of haematology and blood chemistry investigations were all within normal ranges and there were no consistent intergroup differences. At necropsy at termination

of the study there was no evidence of any treatment related macroscopic changes, no intergroup difference in organ weights and no treatment related histopathological changes. In particular there was no indication of any tumorigenic potential of iprodione. The no observable adverse effect level was considered to be in excess of 1250 ppm which was equal to 182 mg/kg bw/day (6).

1.3. Reproductive toxicity

1.3.1. Teratology studies

In a study reported in 1973 groups of female Sprague Dawley rats were given oral doses of 0 (Control), 100, 200 or 400 mg iprodione/kg bw/day between days 5 and 15 of gestation. There was a slight decrease in body weight gain at the high dose level, and the conception rate and mean number of implantations per fertile female were slightly lower at the high dose than in controls. Detailed fetal examinations showed that there were no embryotoxic or teratogenic effects at any dose level. The no observable adverse effect level for maternal toxicity was 200 mg/kg bw/day (7).

In a preliminary dose range finding study reported in 1986, iprodione (purity 94.2%) was administered by oral gavage at dose levels of 0 (Control), 40, 120 240, 400 and 800 mg/kg bw/day to groups of 6 pregnant female Sprague Dawley rats from day 6 to day 15 of gestation. Overt maternal toxicity noted at 400 and 800 mg/kg bw/day consisted of reduced food intake and weight gain and severe clinical signs. One high dose animal died and the others were killed for humane reasons. One rat treated with 400 mg/kg bw/day was killed and only one other animal in this group had viable fetuses. Five late resorptions were seen in one litter from a dam treated with 240 mg/kg bw/day. The dose levels selected for the main study (up to 200 mg/kg bw/day) were therefore considered appropriate (8).

In the main study reported in 1986, iprodione (purity 94.2%) was administered by oral gavage at dose levels of 0 (Control), 40, 90 and 200 mg/kg bw/day to groups of 20 pregnant female Sprague Dawley rats from day 6 to day 15 of gestation. Under the conditions of this test iprodione did not produce any adverse maternal or fetal effects (9, 10, 11).

In a study reported in 1973 groups of female New Zealand White rabbits were given oral doses of 0 (Control), 100, 200 or 400 mg iprodione/kg bw/day between days 6 and 16 of gestation. Maternal toxicity was seen at the high dose (9 of 17 females died during the treatment period) and fetal toxicity was seen at 200 mg/kg bw/day (total resorptions in 3 of 13 females). Detailed examination of the fetal organs and skeletons showed that iprodione was not teratogenic at any of the dose levels tested (12).

In a study reported in 1985, (only a summary of which was submitted for review) iprodione was administered by oral gavage at dose levels of 0 (Control), 20, 60 and 200 mg/kg bw/day to groups of 18 pregnant female New Zealand White rabbits from day 6 to day 18 of gestation. Losses in body weight and decreased food consumption were noted at 200 mg/kg bw/day, during the treatment period. Similar findings, to a lesser extent, were also seen at 60 mg/kg bw/day. Seven abortions and two total litter resorptions were noted at the high dose. No treatment related malformations or developmental variations were seen in any group (13).

1.3.2. Multigeneration studies

In a multigeneration study reported in 1976, (only a summary of which was submitted for review) groups of 10 male and 20 female Sprague Dawley rats received iprodione in the diet at levels of 0 (Control), 125, 250 or 1000 ppm for 5 weeks. Following this initial period, dietary concentrations were increased to 250, 500 and 2000 ppm for the remainder of the premating period and throughout mating, gestation and lactation. This dosing regimen was repeated through 3 generations, with one mating per generation. No evidence of parental toxicity was seen at any dose level and no adverse effects on reproduction were noted. Evidence of toxicity in the pups was noted in the F2 and F3 generation (smaller litter sizes and lower pup weights) at the highest dietary level. No treatment related lesions were observed at limited histopathology of tissues from the F3 generation. The no observable adverse effect level for parents and pups was thus 250/500 ppm, equivalent to approximately 30 mg/kg bw/day (14).

Because of the limitations in the study design of the first experiment, a second multigeneration study was carried out and reported in 1991. In this latter study, groups of 28 male and 28 female Sprague Dawley rats received iprodione (purity 96.2%) by dietary administration at levels of 0 (Control), 300, 1000 or 3000 ppm for 10 weeks prior to mating, and throughout mating, gestation, lactation and weaning of F1a and F1b pups. At mating of the F1a animals (to produce F2a and F2b pups) the high dietary level was decreased to 2000 ppm, since 3000 ppm was considered to exceed the maximum tolerated dose. No adverse effects on reproductive performance were noted in this study. Lower body weight gains (up to 40% below control values) were seen at 3000 ppm, along with a decrease in pup survival. A slightly decreased weight gain was also seen at 2000 ppm and at 1000 ppm. The no observable adverse effect level in this study was thus 300 ppm, equivalent to approximately 21 mg/kg bw/day (15).

Toxicological overview and estimation of ADI

Based upon this review of the available data there is no evidence that iprodione possesses any anti-androgenic potential. This is in contrast to the toxicological properties of pesticides with closely related chemical structures. Vinclozolin has been reported to cause femininization of male rats (16) and procymidone has been reported to cause hormonal imbalance leading to infertility and abnormalities of the male sexual organs and testicular tumours in male rats (17), these findings being reportedly associated with anti-androgenic potential.

In teratology studies in rats and rabbits the NOAEL for maternal toxicity was 200 and 20 mg/kg bw/day respectively. Iprodione was not teratogenic in either species at doses up to 400 mg/kg bw/day.

With regard to estimation of an ADI the crucial no effect level for iprodione is considered to be 18 mg/kg bw/day. This is based on the results of the two dog studies. Although a lower no effect level was derived for the first study, a dietary level of 400 ppm (equal to 18 mg/kg bw/day) is the highest no adverse effect level which is still lower than the actual adverse effect level. This no adverse effect level is further supported by the results of the recent multigeneration study, in which an NOAEL equivalent to 21 mg/kg bw/day was derived.

In view of the fact that the toxicological properties of iprodione do not cause undue concern, it is not necessary to use a safety factor higher than 100 in setting an ADI. The ADI is thus estimated at 0.2 mg/kg bw.

An ADI of 0.2 mg/kg bw is lower than that derived by the JMPR in 1977, but is based on data not available at that time. Although it must be borne in mind that the present review has not considered a complete database, examination of further data (such as subchronic studies in rodents and mutagenicity studies) would not be expected to have any influence on the ADI.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF ALDICARB

(Opinion expressed by the SCP on 26 January 1993)

BACKGROUND AND TERMS OF REFERENCE

Aldicarb is a soil-applied, systemic carbamate insecticide and nematicide used in a wide variety of agricultural crops and in horticulture. In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the toxicity of aldicarb, with a view to establishing an Acceptable Daily Intake and commenting particularly on principles to be used in the risk evaluation for consumer exposure to aldicarb resulting from use on bananas and potatoes.

1. BIOCHEMICAL ASPECTS

1.1. Absorption, distribution and excretion

Aldicarb is readily absorbed, distributed widely in the body and excreted rapidly in mammals. Radiolabeled aldicarb was administered orally to male rats and residues were analyzed over a 14-day period. Excretion of radiolabel in the urine was essentially complete within 4 days (greater than 95% of the administered dose). The major concentrations of metabolites were observed to have been excreted within 24 hours of dosing. Four days following acute oral dosing, residues were not detected in tissues. In dogs, the excretion pattern of aldicarb was similar to that noted following administration in other species. (Refs. 1-6)

1.2. Biotransformation

The metabolic fate of aldicarb has been studied in a variety of animal species. Minor biotransformation differences have been found to occur with respect to quantities of individual matabolites. The basic metabolic profile of aldicarb in all species exmined appears to be the same. Aldicarb is rapidly oxidized to aldicab sulfoxide, a relatively stable metabolite. Aldicarb sulfoxide is slowly degraded by both oxidative and hydrolytic mechanisms yielding the corresponding aldicarb sulfone and sulfoxide oxime. Investigations in plants revealed that aldicarb metabolism proceeds via the same routes in plants and animals. Metabolic transformation in plants is such that 'terminal' residues (to which consumers of treated crops may be exposed) consist largely of the sulfoxide and the sulfone, with, depending on harvest time, a minor residue of parent aldicarb. (Refs. 3, 6-8)

1.3. Effects on enzymes and other biochemical parameters

As noted with other N-methylcarbmate esters, aldicarb is a readily reversible inhibitor of cholinesterase activity and in vitro studies have shown that cholinesterase inhibition induced by aldicarb and its oxidative metabolites (aldicarb sulfoxide and aldicarb sulfone) can be rapidly reversed by simple dilution. (Refs. 7-9)

2. TOXICOLOGICAL ASPECTS

2.1. Acute toxicity

The acute toxicity of aldicarb and its metabolites has been studied in a variety of mammalian species. The acute toxicity of aldicarb is generally similar in different species, oral LD 50's being in the range 0.6-1.5 mg/kg bw and intraperitoneal LD 50's being in the range 0.3-0.7 mg/kg bw. The acute dermal toxicity is much more variable, depending on the vehicle, having greater toxicity in aqueous vehicles and much less toxicity when dry. (Refs. 10-19)

Investigation of the acute toxicity of aldicarb metabolites has shown that aldicarb sulfoxide is of approximately equivalent toxicity to aldicarb itself. However, in all species tested, the sulfone is some 20-50 times less toxic than aldicarb or the sulfoxide and the further degradates (sulfoxide oxime and nitrile) are even less toxic. (Refs. 12, 14, 20-22)

2.2. Short term studies

Mice

In seven day dietary studies, aldicarb induced mortality at 1.2 mg/kg bw/day but a dose of 0.6 mg/kg bw/day was tolerated without substantial effects. A mixture of aldicarb and aldicarb sulfone (1:1) at a dose of 36 mg/kg bw/day did not induce mortality, but severe cholinergic signs of poisoning were observed. Aldicarb sulphone, at a dose of 38 mg/kg bw/day, induced significant body weight decrease but there were no significant organ weight changes. Cholinesterase activity was not determined adequately in any of these studies. (Refs. 23-25)

Rats

In seven day dietary studies in rats aldicarb induced mortality at 16 mg/kg bw/day (7/10) and at 8 mg/kg bw/day (1/10). Rats survived 4 mg/kg bw/day for seven days, with only minor organ weight changes seen at termination. In a 93 day study aldicarb induced mortality at 0.5 mg/kg bw/day, but there were no other consistent treatment-related effects. Cholinestaerase activity was not determined adequately in these studies. (Refs. 26-28)

In another seven day study, no adverse effect levels, based on erythrocyte cholinesterase depression or decreased body weight gain were: aldicarb - 0.8 mg/kg bw/day; aldicarb sulfoxide - 0.4 mg/kg bw/day; and aldicarb sulfone - 2.5 mg/kg bw/day. (Ref. 12)

In a 56 day study rats received aldicarb sulfoxide in the diet at dose up to 1 mg/kg bw/day and aldicarb sulfone at doses up to 16 mg/kg bw/day. There was no mortality, but growth was depressed at the highest dose levels with both the sulfoxide and sulfone. Cholinesterase depression was noted at the high dose levels, but not at lower doses (0.3 mg/kg bw/day sulfoxide, 2.4 mg/kg bw/day sulfone). (Ref. 29)

In a 29-day study aldicarb sulfoxide and sulfone in a 1:1 ratio were administered to rats via the drinking water. Plasma, erythrocyte and brain cholinesterase activitites were depressed at the high dose (1.7 mg/kg bw/day) and the NOAEL was 0.5 mg/kg bw/day. (Ref. 30)

In a series of dietary studies in rats up to 6 months duration carried out to investigate the reversibility of cholinesterase depression induced by aldicarb sulfoxide and sulfone, feeding of control diet for 24 hours completely reversed any cholinesterase inhibition induced by treatment. NOAEL's were 0.125 mg/kg bw/day for the sulfoxide and 0.6 mg/kg bw/day for the sulfone. (Refs. 31-33)

In 7-day dietary studies in rats the NOAEL for aldicarb oxime was 62.5 mg/kg bw/day and the NOAEL for 2-methyl-2-(methylsulfinyl)propanol-1 (a hydrolytic metabolite of aldicarb) was around 500 mg/kg bw/day. (Refs. 21 and 34)

Dogs

A series of dog studies has been conducted to modern standards with aldicarb and aldicarb sulphone. In 2-week dose range finding studies, aldicarb was administered via the diet and the overall NOAEL (based on acetyl cholinesterase inhibition) was around 0.1 mg/kg bw/day. In a one year study the NOAEL was about 0.05 mg/kg bw/day. In similar studies with aldicarb sulphone the NOAEL was about 2.25 mg/kg bw/day. (Refs. 35-40)

Monkeys

As a result of reports of alleged illness associated with consumption of watermelons and cucumbers illegally treated with aldicarb in 1985, two studies were conducted to evaluate the acute toxicity of aldicarb residues in food commodities. Two crops were chosen for the studies: bananas and watermelon. Each study was performed using three male and three female Cynomolgus monkeys, with an additional group of three males and three female monkeys serving as a control (total of twelve animals per study). Following analytical determination of actual residues in the two commodities to be consumed, (approximately 0.3 ppm in bananas and 5 ppm in watermelons), intake of the treated crops was adjusted with control fruit to provide a dose of exactly 0.005 mg/kg bw aldicarb equivalent. The animals were offered the fruit as the first meal of the day, and consumed it immediately. In both studies, there was no evidence of acute cholinergic distress or any other signs of toxicity in any animal. Depression of plasma cholinesterase activity was evident within one to four hours after ingestion. Erythrocyte cholinesterase activity was not depressed in either study. Rapid recovery of all enzyme activity was observed. (Refs. 41 and 42).

2.3. Long term/Carcinogenicity studies

Mice

Groups of 50 male and 50 female B6C3F1 mice (25 of each sex were used as controls) were administered aldicarb in the diet at dietary levels of 0, 2 and 6 ppm for 103 weeks in a carcinogenicity bioassay. There was no mortality noted attributable to aldicarb in the diet. Gross and microscopic examination of tissues, organs and all gross lesions was performed and it was concluded that aldicarb was not carcinogenic for the B6C3F1 strain of mice of either sex. (Ref. 43)

Groups of 44 male and 44 female CD-1 mice were fed aldicarb in the diet to achieve dosage levels of 0, 0.1, 0.2, 0.4 and 0.7 mg/kg bw/day for 18

months. Mortality was evident in males at the two highest dosage levels and in females at the three highest dosage levels during the first two and a half months of the study. Following this period, aldicarb was admixed with the diet in a different manner which appeared to eliminate its acutely toxic effects. At the high dosage level in males there was a statistically significant increase in hepatomas found predominantly in the survivors at the termination of the study. An increase in lymphoid neoplasias occured in the mice that died, but none of the male mice surviving at the end of the study were found to have lymphoid neoplasias. There were no significant increases in any other types of tumors at dosage levels of 0.4 mg/kg and below. (Ref. 44)

Groups of 50 male CD-1 mice were fed aldicarb in the diet to achieve dosage levels of 0, 0.1, 0.3 and 0.7 mg/kg bw/day in an effort to verify the results of the previous mouse carcinogenicity bioassay. A group of 150 mice were used as concurrent controls with a mouse being sacrificed for each treated animal that died during the course of the study. Diets were prepared by dissolving aldicarb in acetone and mixing the solution with the diet. The aldicarb was the same sample as used in the previous study and the duration of the study was approximately the same an in the previous trial. There was no mortality observed in the study as a result of aldicarb in the diet. At the end of 18 months cumulative mortality at all dosage levels was the same as noted in controls. There was no effect of aldicarb on growth in any of the groups. There was no significant association between aldicarb in the diet and the formation of tumors, particularly with respect to the incidence of hepatomas, lung adenomas, and lymphoid neoplasias. It was concluded that the administration of aldicarb at levels up to and including 0.7 mg/kg bw/day for approximately 18 months did not result in a higher than normal incidence of tumors and the inclusion of aldicarb in the diet of CD-1 mice did not result in an increased incidence of carcinogenic response. (Ref. 45)

Groups of Charles River CD-1 mice (50/sex/group) were administered 0, 0.15, 0.6, 2.4 and 9.6 mg/kg aldicarb sulphone via the diet for 18 months. Aldicarb sulphone did not affect tumour incidence or produce any pathological alteration in this strain of mouse. (Ref. 46)

Rats

Groups of 20 male and 20 female rats were fed aldicarb for two years in the diet to achieve dosage levels of 0, 0.005, 0.025, 0.05 and 0.1 mg/kg bw/day. There was no mortality over the course of the study attributable to the presence of aldicarb in the diet. Growth was normal at all dosage levels as was consumption of food and behavioural characteristics. Blood and brain cholinesterase activity, measured at 6 and 12 months, were normal. Microscopic examination of tissues and organs for histopathologic occurrences and neoplasms showed the incidence of lesions to be similar in aldicarb-treated and in control groups. (Ref. 47)

Groups of 50 male and 50 female F344 rats (25 of each sex were used as controls) were administered aldicarb in the diet at levels of
0, 2 and 6 ppm for 103 weeks. There was no mortality noted attributable to aldicarb in the diet. Gross and microscopic examination of tissues, organs and all gross lesions was performed and it was concluded that aldicarb was not carcinogenic for the F344 strain of rat of either sex. (Ref. 43)

Groups of 20 male and 20 female rats were fed aldicarb for two years in the diet to achieve dosage levels of 0 or 0.3 mg/kg bw/day. In addition, groups of rats were fed aldicarb sulfoxide (0.3 or 0.6 mg/kg bw/day), aldicarb sulphone (0.6 or 2.4 mg/kg bw/day) or a 1:1 mixture of aldicarb sulphoxide and aldicarb sulphone (0.6 or 1.2 mg/kg bw/day). In the initial

phases of the study, there was a slightly higher mortality noted in the high dosage level of aldicarb sulfoxide and in the group receiving the combined aldicarb sulfoxide and aldicarb sulfone. A slight increase in mortality was also noted at the latter part of the study with aldicarb sulfoxide. Growth was slightly depressed at the high dose level of the sulfoxide:sulfone mixture, primarily in males. There were no apparent effects on growth with respect to the aldicarb, aldicarb sulfoxide or aldicarb sulfone administered alone. Gross and microscopic examination of tissues and organs showed that sporadically distributed lesions were not considered to be indicative of damage induced by aldicarb, its major metabolites, or the combintion of the sulfoxide and sulfone and there was no evidence of carcinogenicity. (Ref. 48)

2.4. Reproduction studies

2.4.1. Multigeneration studies

Groups of rats (8 male and 16 female rats per group) were administered aldicarb in the diet to achieve doses of 0, 0.05 and 0.1 mg/kg bw/day for approximately 90 days and mated to initiate a 3 generation, one litter per generation reproduction study. In addition to the reproduction indices (fertility, gestation, viability and lactation) the F3 generation was maintained for an additional period and tissues from these animals were histologically examined at either weaning or at 90 days of age. There was no effect on reproductive performance and body weights of both male and female pups at weaning were similar to control values as were results of gross and microscopic examinations of tissues and organs in the F3 weanling and 90 day old animals. (Ref. 49)

Groups of rats (10 male and 20 female per group) were administered aldicarb in the diet to achieve doses of 0, 0.2, 0.3 and 0.7 mg/kg bw/day for 100 days and mated to initiate an additional 3 generation (one litter per generation) reproduction study. A larger group was used for the F2 generation (15 male and 25 female rats) as male pups of this generation were maintained on aldicarb diets for 148 days and subjected to a dominant lethal bioassay. Each female in the group was mated with 2 treated males and allowed to maintain pregnancy until day 12 when they were sacrificed and examined. At the high dose level, body weights of both male and female F2 pups were lower than the control values. Overall, there were no effects on any of the reproduction indices (fertility, gestation, viability, or lactation). Gross and microscopic examinations of the parents and pups of the high level and control groups showed no effects attributable to aldicarb. (Ref. 50)

Aldicarb sulphone (99.76% pure) was administered to groups of 10 male and 20 female rat via the diet to achieve dosages of 0, 0.6, 2.4 and 9.6 mg/kg bw/day for approximately 100 days. Rats were then mated to initiate a three generation, one litter per generation, reproduction study. Male rats fed 9.6 mg/kg bw/day exhibited reduced body weights. There were no differences from the control with regard to fertility, gestation survival or viability indices. It was determined that aldicarb sulphone, at levels up to and including 9.6 mg/kg bw/day, was without adverse effects on reproduction under the conditions of this study. (Ref. 51)

2.4.2. Teratogenicity studies

Rats

Pregnant Sprague-Dawley rats were given aldicarb by gavage at levels of 0, 0.125, 0.25 and 0.5 mg/kg bw/day on gestational day 6 through day 15. Maternal toxicity was indicated, at the highest dose, by reduced weight

gain and food intake during the treatment and post treatment period as well as by the occurrence of three maternal deaths and at the two highest levels by reduced food consumption. Gestational parameters, including ovarian corpora lutea, number of implantations per litter and sex ratio were unaffected by treatment. Foetal body weight per litter was significantly reduced at 0.5 mg/kg bw/day. There was no increase in the incidence of external or skeletal malformations. A significantly increased incidence of dilated lateral ventricles of the brain was observed only at 0.5 mg/kg bw/day. No increased incidence of malformation was observed in the absence of clear maternal toxicity nor were these malformations accompanied by more severe malformations. The no-observed effect level was 0.125 mg/kg bw/day for maternal toxicity and 0.25 mg/kg bw/day for fetotoxicity. (Ref. 52)

The aldicarb sulphone reproduction study incorporated a teratology bioassay. The animals were divided into four groups and orally dosed with either 0, 0.6, 2.4 or 9.6 mg/kg bw/day at one of the following time intervals of gestation: 0-20 days, 6-15 days, or 7-9 days. All animals were sacrificed before parturition (day 20) and the foetuses examined for skeletal and visceral changes. Malformations were essentially non-existent and there was no indication of structural abnormalities produced under the conditions of the study at levels up to and including 9.6 mg/kg bw/day. (Ref. 51)

Rabbit

Pregnant Dutch Belted rabbits (16/dose) were dosed by oral gavage at 0.1, 0.25, and 0.5 mg/kg bw/day on days 7 to 27 of gestation. Signs of maternal toxicity in the treated groups included small amount of faeces, pale kidneys and hydroceles on oviducts. A loss in body weight occurred in the 0.25 and 0.5 mg/kg bw/day dosage groups during gestation days 7 to 27. A decline in the number of viable foetuses per doe was observed in all treatment groups (8.7, 5.0, 6.5 and 6.2 for the control, 0.1, 0.25 and 0.5 mg/kg bw/day treatment groups respectively). This can be explained by an unusually high number of implantations in the control group (9.8, 6.1, 7.2 and 7.8 implantations per doe in the control, 0.1, 0.25 and 0.5 mg/kg bw/day treatment groups respectively). There was clearly no dose-effect relationship. There were also no increases in post-implantation losses and there was no meaningful difference in any of the other developmental parameters nor in the number of developmental variations or malfunctions between the control and treatment groups. Aldicarb did not produce a teratogenic response when administered orally to rabbits by gavage at dosage levels up to 0.5 mg/kg bw/day. (Ref. 53)

2.5. Mutagenicity studies

Results of genotoxicity tests are summarised in Table 1 and Table 2 (for in vitro and in vivo tests with aldicarb) and Table 3 (for in vitro tests with aldicarb metabolites.

Table 1: Results of in vitro genotoxicity assays on aldicarb

Test System	Test Object	Concentration	Purity	Results	Reference
Ames test	S Typhimurium TA98,TA100,TA1535, TA1537,TA1538	50-5000 µg/plate	nk	Negative	54
Reverse mutation assay	E.Coli WP2	nk	nk	Negative	55
Reverse mutation assay	S Cerevisiae	nk	nk	Negative	56
HGPRT forward mutation assay	CHO cells	1000-5000 µg/ml	nk	Negative	57
SCE mutation assay	Human lymphocytes	nk	nk	Weak Positive	58
Cytogenetics mutation assay	Human lymphocytes	10-250 µg/ml	nk	Weak Positive	59
UDS	Rat hepatocytes	0.16-5000 µg/ml	nk	Negative	60
DNA damage	S Typhimurium TA 1538 uvrB- TA 1978	>500 µg/disc	nk	Positive	61

nk: not known

Table 2: Results of in vivo genotoxicity assays on aldicarb

Test System	Test Object	Concentration	Purity	Results	Reference
Micronucleus test	Mouse	0.001-0.01 mg/kg bw	93.47%	Negative	62
Micronucleus test	ICR Mouse	0.1-0.4 mg/kg bw	99.7%	Negative	63
Dominant lethal test	Wistar rat	0.2-0.7	99.2%	Negative	50

Table 3: Results of in vitro genotoxicity assays on aldicarb metabolites

Test System	Test Object	Concentration	Purity	Results	Reference
Ames test	S Typhimurium TA98,TA100,TA1535, TA1537, TA1538	50-5000 µg/plate Sulphoxide	nk	Negative	64
Ames test	S Typhimurium TA98,TA100,TA1535, TA1537, TA1538	100-10000 µg/plate Sulphone	nk	Negative	65
HGPRT forward mutation assay	CHO cells	500-1500 µg/ml Sulphone	nk	Negative	66
Cytogenetics mutation assay	CHO cells	50-500 µg/ml Sulphone	nk	Negative	67
UDS	Rat hepatocytes	0.1-3000 µg/ml Sulphone	nk	Negative	68

nk: not known

2.6. Investigative studies on immunotoxicity

Aldicarb was evaluated for its ability to modulate the immune response in two strains of mice. The B6C3F1 mouse was chosen because it is the strain used by the U.S. National Toxicology Program for immunotoxicology studies, and the hybrid Swiss Webster mouse was included because aldicarb had been reported to suppress the splenic plaque-forming cell response to sheep red blood cells in an inverse dose-response fashion (Ref. 69). An attempt was made to reproduce these findings and to expand the data base using more standardised techniques with the B6C3F1 strain. Aldicarb was administered in the drinking water ad libitum for 34 consecutive days to female mice of both strains at dosages ranging from 0.1 to 1,000 ppb in 10-fold increments (equivalent to 0.04 to 364 mg/kg bw/day). Aldicarb had no effects on body weights or organ weights, on numbers or types of circulating white blood cells, or on the microscopic pathology of the thymus, spleen, liver, kidneys, or lymph nodes, or on measures of immune function. (Ref. 70)

In another study, adult female B6C3F, mice received distilled water only or water containing 1.0, 10 or 100 ppb of aldicarb daily for 34 days. To further develop an immune profile of the compound, following aldicarb exposure, the ability of splenic natural killer (NK) cells as well as specifically sensitized cytotoxic T-lymphocytes to lyse YAC-1 lymphoma and, P815 tumor cells, respectively, were evaluated. The results of this study demonstrated that aldicarb did not impact upon the functional ability of interferon-induced splenic NK cells to lyse YAC-1 lymphoma target cells. In addition, aldicarb had no significant effect on body weights, on spleen cellularity or cell viability or on absolute weights of lymphoid organs. The absence of statistically significant effects on any of these parameters indicated that aldicarb did not have adverse effects on the immune system of mice in this experiment. (Ref. 71)

2.7. Observations in humans

Groups of 4 adult male volunteers were administered aldicarb orally in aqueous solution at dosage levels of 0.025, 0.05 and 0.1 mg/kg body weight. Clinical signs of reaction to treatment were recorded and whole blood cholinesterase activity was measured up to six hours after administration of the sample. Total urine voided was collected and aldicarb-excretion patterns for the initial eight hours after dosing were evaluated. In addition, spot samples were taken at 12 and 24 hours. Acute signs, typical of anticholinesterase agents, were observed at the high dose level within one hour after administration of aldicarb. There were no signs of reaction to treatment observed at the 0.05 mg/kg body weight dose level. Cholinesterase depression was observed in all volunteers prodominantly within 1-2 hours after treatment. Within the first six hours of treatment almost all cholinesterase depression and clinical signs were diminished. Examination of urinary excretion patterns showed that approximately 10% of the administered dose was excreted as carbamates (toxic residues) within the first eight-hour interval. Cholinesterase analyses confimed the same rapid inhibition and recovery pattern with man as had been observed in experimental animals. (Ref. 72)

In another study, two additional subjects were administered aldicarb in water solution at dosage levels of 0.05 and 0.26 mg/kg body weight. Acute signs of poisoning were recorded at the higher dose level and atropine was administered to aid recovery. No signs of poisoning were recorded with the lower dose level. Urinary excretion of carbamate residues within 24 hours accounted for approximately 10% of the administered dose. (Ref. 73)

A double blind, placebo controlled study has ben conducted, in which aldicarb was given as a single oral dose to healthy male and female subjects. The doses administered were: placebo (22 subjects - 16 males and 6 females), 0.01 mg/kg bw (8 males), 0.025 mg/kg bw (8 males and 4 females), 0.05 mg/kg bw (8 males and 4 females) and 0.075 mg/kg bw (4 males). Subjects were screened before entry by general medical history and examination and laboratory tests including haematology, clinical chemistry and urinalysis Clinical measurements were made at intervals before and after dosing. These included vital signs, (systolic and diastolic blood pressure, pulse rate), pulmonary function tests, pupil size, electrocardiographs, salivation and clinical signs of nausea, vomiting, diarrhoea, sweating, abdominal cramps, involuntary movement and slurred speech. Samples were taken for urinalysis, clinical chemistry (including red blood cell and plasma cholinesterase activity) and haematology evaluation before and after dosing. Urine and blood were collected for aldicarb analysis. The results of these latter analyses were not reported. There were no clinically significant changes in vital signs, pupil size, pulmonary function, ECG's, salivation, clinical signs, clinical chemistry (apart from cholinesterase), haematology or urinalysis in the study. Cholinesterase activity in red cells and plasma was maximally depressed at 1 hour after dosing and had recovered by 8 hours in all subjects. The fall in activity was dose related. Only marginal depression of cholinesterase activity (<20%) was seen in erythrocytes from patients treated with 0.01 or 0.025 mg/kg bw and in plasma at 0.01 mg/kg bw. Depression in cholinesterase activity >20% was seen in erythrocytes at 0.05 and 0.075 mg/kg bw and in plasma at 0.025, 0.05 and 0.075 mg/kg bw. No serious adverse events occurred. The minor adverse events which were recorded were similar to those reported in other volunteer studies. Only one subject (0.075 mg/kg bw group, actual dose 0.06 mg/kg bw) developed symptoms which were reported to be related to aldicarb. The meeting concluded that the no adverse effect level in this study should be based on depression (>20%) in erythrocyte cholinesterase activity. The NOAEL was thus 0.025 mg/kg bw. (Ref. 74)

Several misuses of aldicarb on watermelons and cucumbers have been reported in the literature. Goes, et al, (1980, Ref. 75) reported on a suspected food-borne intoxication in Nebraska associated with the consumption of aldicarb contaminated hydroponically grown cucumbers. Two episodes were reported (April 1977: 9 cases; July 1978: 5 cases). Neither cholinesterase determinations nor urine residue determinations were performed in any of these patients. Four cucumbers (two in the supermarket and two in the greenhouse were analysed and aldicarb content was 6.6 and 10.7 ppm (supermarket) 0 and 9.9 ppm (greenhouse). No data are available on cucumbers actually eaten and no attempts to correlate symptoms and aldicarb consumption were made.

Hirsch, et al, (1987, Ref. 76) reported over 300 alleged intoxications in Canada following ingestion of aldicarb contaminated cucumbers (residue levels up to 26 ppm). In only five cases involving 13 patients, were any remaining portion of the cucumbers consumed still available for analyses.

Goldman (1990, Ref. 77) reported on four outbreaks of aldicarb poisoning having occurred in California and other states in the USA from 1978 to 1988.

Outbreak 1 : watermelons, 1985
Outbreak 2 : watermelons, 1987
Outbreak 3 : cucumbers, 1988
Outbreak 4 : cucumbers, 1978 (corresponds to Ref. 75)

In the first outbreak, 1376 people were allegedly ill due to consumption of contaminated watermelons. Of this total, 308 were considered by the authors themselves as unlikely (235) or incomplete (73). From the remaining cases only 28 were supported by positive findings of aldicarb residues in the food commodity. All the other cases were only supported by self diagnosis.

An epidemiologic study of potential adverse health effects of aldicarb metabolites in drinking water was made by the South Carolina Pesticides Hazard Assessment Program Center. In two surveys conducted on Long Island, New York, the major cohorts were selected on the basis of aldicarb levels in their drinking water. Levels of contamination were generally 4-12 ppb, but a maximum of 400 ppb was reported. Questionaires to determine water and food consumption, symptoms experienced, and diagnosed illnesses were sent to 1,035 residents of 462 households. Although the initial survey indicated a possible association between the incidence of diarrhea and levels of aldicarb in the water, the follow-up study, focusing on children, did not confirm this association. No relationship was detected between food consumption or water source and adverse health symptoms, and self-reported physician-diagnosed illnesses for 1974-1979 were not significantly related to levels of aldicarb in the water. (Ref. 78)

Another survey attempted to relate self-reported symptoms suggestive of peripheral neuropathy to aldicarb levels in drinking water in Suffolk County, New York. The response rate was less than 20%. Responses were classified as "probably", "possibly", or "vaguely" suggestive of a neurologic syndrome. A significant correlation with aldicarb concentration was obtained only by combining all three categories of response, including reports of just one symptom or of symptoms not forming any cohesive syndrome. The authors concluded only that further study was needed. (Ref. 79)

A pilot epidemiological study by Fiore, et al, (1986, Ref. 80) evaluated a wide range of clinical immunological parameters in 23 women exposed to aldicarb in their drinking water and in a non-exposed control group. The groups did not differ in any immunological parameters except in T8-Lymphocytes: the number was considered as elevated in five exposed and in one control. Observation of an elevated stimulation assay response to one of the large number of antigens (Candida) was not considered as toxicologically significant, nor was it attributed to aldicarb exposure.

In a follow up study, five women of the preceeding group and still exposed to aldicarb were examined versus previous control women plus women previously exposed but for whom exposure no longer occurred (e.g. change in water supply due to addition of a charcoal filter). According to the authors, this follow-up study confirmed the previous results, except for increased response to Candida antigens which was no longer present. (Ref. 81)

TOXICOLOGICAL OVERVIEW AND ESTIMATION OF ADI

Aldicarb is a carbamate insecticide which inhibits acetylcholinesterase in a quickly reversible manner. Following administration to animals, aldicarb is rapidly absorbed, widely distributed in the body and rapidly excreted. Metabolism appears to be similar in all animal species and in plants, aldicarb being rapidly metabolised to aldicarb sulphoxide, which is more slowly degraded to aldicarb sulphone. Consumers of treated crops are exposed to residues which consist largely of the sulphoxide and the sulphone, with a minor residue of parent aldicarb.

Aldicarb has high acute toxicity in a wide variety of mammalian species and the signs of toxicity are those commonly associated with acetylcholinesterase inhibition. Although aldicarb sulphoxide is of approximately equivalent acute toxicity to aldicarb itself, aldicarb sulphone is some 20 to 50 times less acutely toxic than either aldicarb or the sulphoxide.

Short term and long term studies have been performed in rats, mice and dogs with aldicarb and aldicarb metabolites, both alone and in combination. As would be expected, acetylcholinesterase depression is the most significant indicator of toxicity that can be evaluated. However, this cholinesterase depression is quickly reversible and careful attention must be paid to the methods of sample collection and determination of cholinesterase activity. Continuous administration of aldicarb to the test animals until collection of samples for analysis is important, as is rapid analysis under carefully controlled conditions. Since these precautions were not followed in many of the earlier repeat dose studies, it is considered inappropriate to utilise NOAELs from these studies in derivation of an ADI. In acceptable one year dog studies performed to modern standards, NOAELs (based on acetylcholinesterase inhibition) were around 0.05 mg/kg bw/day for aldicarb and 2.25 mg/kg bw/day for the sulphone.

Carcinogenicity studies in rats and mice with aldicarb and its metabolites revealed no indication of any carcinogenic potential. Similarly, from the results of a wide variety of genotoxicity tests, it was concluded that aldicarb is not genotoxic.

In multigeneration studies in rats there were no effects on reproductive performance at the highest doses tested of 0.7 mg/kg bw/day aldicarb or 9.6 mg/kg bw/day aldicarb sulphone. Aldicarb did not display any teratogenic potential in rats or rabbits in studies which included maternally toxic doses.

There was no evidence for immunotoxicity of aldicarb in mice in a number of functional assays of cell mediated immunity and in host resistance to respiratory infection. Epidemiological studies provided no evidence that aldicarb could significantly alter immunological parameters in man.

The anticholinesterase potential of aldicarb has been extensively investigated in humans. These studies revealed the same pattern of rapid blood cholinesterase inhibition and rapid recovery seen in experimental animals. In a recent double-blind placebo-controlled study, transient erythrocyte cholinesterase depression was seen at 0.05 mg/kg bw, and the NOAEL for cholinesterase depression (discounting changes in plasma enzyme only) was 0.025 mg/kg bw.

There have been a number of food-related aldicarb poisoning incidents in humans reported in the literature. These have all been associated with misuse of aldicarb and reliable quantification of the dose of aldicarb involved has always proved difficult, if not impossible. It was concluded that these reports provide no reason to discount the results of controlled experiments in animals and man.

An ADI may be allocated using a 10-fold safety factor applied to the no observed adverse effect level for depression of erythrocyte cholinesterase activity in human volunteers, giving an ADI of 0.003 mg/kg bw.

In comparing this ADI with consumer exposure to aldicarb residues there are a number of important factors which must be borne in mind:

- The toxicity of aldicarb is quickly reversible, and there are no known cumulative effects. Consumer exposue therefore amounts to repeated acute exposures and it follows that food intake figures used in exposure estimations should be based on single "meal-sized" portions, rather than average "life-time" figures.

- For the same reason, since it is possible that residues of aldicarb and its metabolites in individual tubers/fruits can be variable, relevant residues data, based on individual tuber/fruit measurements, rather than overall bulk estimates, should be used in estimation of consumer exposure to aldicarb and its metabolites.

- The identity of the exact residue is important. Measurements usually combine parent aldicarb with the sulphoxide and the sulphone. Where exposure to this total residue exceeds, or is close to the ADI, differentiation of the sulphone could provide valuable information, since the sulphone is some 20 to 50 times less toxic than aldicarb or the sulphoxide.

- A brief review of the available residues data has indicated that the required level of detail may currently not be available. However, it is likely that the sulphone is a major component of the residue in foodstuffs, particularly in potatoes and bananas where agricultural practice generally involves long intervals between application and harvest. Therefore, where consumer exposure is close to the ADI, it is considered appropriate to allow use of aldicarb to continue while the required residue data are generated.

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SUPPLEMENTARY REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF ALDICARB
(Residue intake resulting from consumption of potatoes and bananas)

(Opinion expressed by the SCP on 26 January 1995)

BACKGROUND AND TERMS OF REFERENCE

Aldicarb is a soil-applied, systemic carbamate insecticide and nematicide used in a wide variety of agricultural crops and in horticulture. In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the toxicity of aldicarb, with a view to establishing an Acceptable Daily Intake and commenting particularly on principles to be used in the risk evaluation for consumer exposure to aldicarb resulting from use on bananas and potatoes. This work was published in the Committee's opinion expressed on 26 January 1993.

After publication of this document the Commission requested the Scientific Committee for Pesticides to review the potential consumer exposure to aldicarb resulting from use on bananas and potatoes, and comment on these intakes in relation to the Acceptable Daily Intake proposed in the Committee's opinion of 26 January 1993.

1 RESIDUE LEVELS

There are proposed EU MRLs for aldicarb on potatoes, at 0.5 mg/kg, and bananas, at 0.3 mg/kg. However, as stated in the Committee's previous opinion, it is possible that these levels do not represent the correct residue levels to use for risk assessment purposes. This is because the MRLs are based on analysis of residue levels in bulk samples of commodity and it is possible that residues of aldicarb and its metabolites in individual tubers or fruits have a greater variability than for bulk samples. Since the toxicity of aldicarb is acute and quickly reversible, it is important to be able to calculate the residue intake resulting from specific exposure events. Relevant residues data to perform these intake estimations should therefore be based on individual tuber or fruit residue levels.

As stated in the Committee's previous opinion, the exact identity of the residue may be important. Residue determinations usually combine parent aldicarb with the sulphoxide and sulphone metabolites. Although the sulphoxide is of similar toxicity to aldicarb itself, the sulphone is some 20 to 50 times less toxic. Differentiation of any sulphone component in the residue could therefore provide valuable additional information for risk assessment purposes.

1.1 Residue levels in potatoes

Application rates for aldicarb by broadcast appplication on potatoes vary widely in different member states (e.g. Belgium 1 kg/ha, Spain and Netherlands 3 kg/ha, UK and Ireland 3.36 kg/ha), all applications are at planting. As an alternative to these broadcast applications, there is some aldicarb applied 'in furrow' at planting in the UK, at a rate of up to 12.8 g/100m. Based on these application rates and considering that other differences in agricultural practice and climate ought not to affect residue levels markedly, it is concluded that agricultural practice in the UK ought to give rise to the highest residues. Assessments of consumer intake can therefore be based on residues data relevant to the UK conditions of use.

Results from trials in the UK revealed that residues in composite samples were 0.3-0.35 mg/kg for early potatoes treated by broadcast application, or <0.05-0.1 mg/kg for maincrop potatoes using in furrow treatment. These data support the MRL of 0.5 mg/kg. Where analysis of individual tubers was carried out (in early potatoes), residues were detected at levels of 0.02-0.76 mg/kg. Where samples were analysed for the individual components of the residue, no residue of aldicarb was detected and the residue consisted of approximately 78% aldicarb sulphoxide and 22% aldicarb sulphone.

Potatoes are a commodity which are never eaten in the raw state, and thus it is valid to consider the possibility of reductions due to cooking or processing. Processing and cooking studies have been conducted with potatoes. The results showed that residues will degrade (compared to the raw agricultural commodity) depending on the procedures utilised. Results of these studies showed that aldicarb residues are reduced in preparation of potato chips (crisps) (17-99%), French fries (25-44%), hash browns (70-76%), canning (50-80%), oven baking (53-80%) and boiling (18-88%). Washing and peeling reduced residues by 55-100%. Commercial abrasion peeling reduced residues by 15-36%. Microwave baking showed only minor or no reduction. The great variability in these reduction factors hampers their general application in risk assessment.

1.2 Residue levels in bananas

In contrast to the agricultural practice in potatoes, aldicarb is applied to bananas around the base stem of the growing plant. Residues in banana fruits arise from root uptake, followed by transfer via sap and distribution within the plant. Consequently the residues are more closely related to the rate applied and the time between treatment and harvest. Agricultural practice in different member states is similar with application rates of 2g per tree and pre-harvest intervals of 100-120 days. Results from relevant trials revealed that residues in composite samples were 0.05-0.37 mg/kg. These data support the MRL of 0.3 mg/kg. The registrant has recently proposed changing this pre-harvest interval, to a minimum of 180 days and they propose that this modification leads to a change in the MRL from 0.3 mg/kg to 0.1 mg/kg. Very few trials are available for a pre-harvest interval of 180 days, but the limited data available revealed residues generally around 0.01 to 0.07 mg/kg, supporting an MRL of 0.1 mg/kg. The registrant recognises that the residues results on bananas at the new proposed pre-harvest interval of 180 days is limited and they are reportedly in the process of developing protocols to conduct additional trials to generate further data to support this extended pre-harvest interval.

The variability of residues in bananas taken individually has been considered in some residue trials. The results showed minimal variability between different fruits from the same bunch, but more variability between different bunches. The results indicate that the residue concentration in individual banana samples is never more than about 2.25 times the residue concentration determined for composite samples. In the recent residue trials the analytical methodology permitted separate quantification of parent aldicarb and the sulphoxide and sulphone metabolites. These results show that the residue is essentially composed largely of the sulphoxide. The sulphone is occasionally detected and parent aldicarb is virtually never found above the quantification limit.

Processing and cooking trials are not applicable for a commodity such as bananas, which are essentially always consumed raw. However, it is important to look at the variation in the residue level between the fruit pulp and the peel, since the peel is hardly ever consumed. Relevant residues data are available and show that there is a trend for the residue concentration in the peel to be higher than that in the pulp, but these differences are small. For the purposes of consumer risk assessment it may be assumed that the concentration in the pulp is the same as that determined in the whole fruit.

2 CONSUMPTION OF POTATOES AND BANANAS

As stated in document 6254/VI/92 the toxicity of aldicarb is quickly reversible, and there are no known cumulative effects. Consumer exposure therefore amounts to repeated acute exposures and it follows that food intake figures used in exposure estimations should be based on single "meal-sized" portions, rather than average "life-time" figures. The following evaluation is directed towards assessing which consumption habit would be expected to lead to the highest intake of residues in different sectors of the general population and then evaluating the relevant data available for that situation.

2.1 Consumption of potatoes

It is evident from the data presented in section 1.1 that processing and cooking reduction factors have a large effect on the intake of aldicarb residues from potatoes. Microwave baking has no associated reduction factor, and hence it follows that this situation should lead to the highest residue intake. Microwave baking should be considered to be used for all age groups of the population, but is restricted to maincrop potatoes.

New potatoes are also consumed by all age groups of the population, these generally have slightly higher residues than maincrop potatoes, but are generally always cooked by boiling, with an associated reduction factor. 'Microwave boiling' (microwave cooking using a small quantity of water in a sealed container) is a preparation method increasingly being used for new potatoes. No reduction factor data are available for this preparation method.

Typical consumption values (taken from UK MAFF food portion size database) are presented below:

Baked potatoes (with skin):	Small:	100g
	Medium:	180g
	Large:	220g

Boiled new potatoes:	Small portion:	120g (3 average tubers)
	Medium portion:	175g (4-5 average tubers)
	Large portion:	220g (5-6 average tubers)

In this assessment it has been assumed that small portions of potatoes are consumed by infants, that a medium baked potato, or medium portion of boiled new potatoes might be consumed by a 30 kg child, while the large portion sizes are restricted to 60 kg adults.

2.2 Consumption of bananas

There are no reduction factors relevant for the consumption of bananas treated with aldicarb and bananas are consumed by all age groups of the population. Bananas are, however, always peeled prior to consumption, so the consumption figures must relate to weight without skin. Typical consumption values (taken from UK MAFF food portion size database) are presented below:

Bananas (without skin):	Small:	80g
	Medium:	100g
	Large:	120g

In this assessment it has been assumed that a two large bananas might be consumed at a single sitting by an adult, child or infant.

3 ACCEPTABLE INTAKES OF ALDICARB

The ADI for aldicarb, an anticholinesterase carbamate insecticide, has been established at 0-0.003 mg/kg bw. This is based on the NOAEL for erythrocyte acetylcholinesterase depression in a single dose, double blind, placebo controlled, human volunteer study, which was 0.025 mg/kg bw.

When performing risk assessments for 'acute' or 'meal sized' consumer exposure it has been questioned whether the ADI (which assumes daily exposure throughout life) is the correct toxicological comparison. It has been argued that single doses might be tolerated at higher dose levels than multiple repeated doses, or that different aspects of the toxicological database may become crucial when only short term administration is considered.

Aldicarb was found not to be carcinogenic or teratogenic and did not cause toxicities other than the cholinergic syndrome. In contrast to many other pesticides, a single dose human volunteer study is considered appropriate for use in establishing the ADI since the cholinesterase inhibition caused by aldicarb is rapidly reversible and any chronic exposure to aldicarb may be considered as a series of repeated acute exposures. The establishment of an acute reference dose for aldicarb, in addition to the ADI, is considered below, by reference to data summarised in previous evaluations of aldicarb performed by the SCP and by the JMPR.

3.1 Animal data on acute and short term toxicity of aldicarb and aldicarb metabolites

The acute oral LD_{50} of aldicarb is around 0.5-1.5 mg/kg bw in rats, mice, guinea pigs and rabbits. There are no data in other species and from the available data it is not possible to

establish effect and no effect dose levels. Aldicarb sulphoxide has a similar acute toxicity to aldicarb itself, but the acute oral LD_{50} of aldicarb sulphone is around 20-75 mg/kg bw.

In short term dietary administration (up to 7 days) rats can tolerate dietary levels of aldicarb equivalent to 4 mg/kg bw/day without mortality. Investigations of cholinesterase activity in these rat studies were considered not adequate to determine effect and no effect levels. In two small 2 week studies in beagle dogs (which included acceptable investigations of cholinesterase activity), the overall no adverse effect level for clinical signs of cholinergic toxicity and cholinesterase inhibition was 10 ppm (0.32 mg/kg bw/day). Erythrocyte cholinesterase was inhibited at 10 ppm (>25%), but brain cholinesterase remained unaffected. In a similar study with aldicarb sulphone, the no adverse effect level for clinical signs of cholinergic toxicity was 100 ppm (3.2 mg/kg bw/day) while the no adverse effect level for brain cholinesterase activity was 30 ppm (0.96 mg/kg bw/day).

Groups of 3 male and 3 female *cynomolgus* monkeys were fed treated fruit (bananas and watermelons) to give a single dose of 0.005 mg aldicarb/kg bw. There were no clinical signs of reaction to treatment and although plasma cholinesterase activity was depressed by about 35%, erythrocyte cholinesterase activity remained undisturbed.

3.2 Human data on acute toxicity of aldicarb

Groups of 4 adult male volunteers were administered aldicarb orally in aqueous solution at dose levels of 0.025, 0.05 or 0.1 mg/kg bw. Clinical signs of cholinergic intoxication were observed at 0.1 mg/kg bw, within 1 hour of dosing and whole blood cholinesterase activity was depressed in all subjects. All cholinesterase depression and clinical signs of reaction to treatment reversed within 6 hours of dosing.

In another study 2 subjects were administered aldicarb orally in aqueous solution at dose levels of 0.05 or 0.26 mg/kg bw. Acute signs of poisoning were recorded at the higher dose level.

In a double blind, placebo controlled study aldicarb was given as single oral doses to groups of male and female volunteers, as follows: placebo, 16 males and 6 females; 0.01 mg/kg bw, 8 males; 0.025 mg/kg bw, 8 males and 4 females; 0.05 mg/kg bw, 8 males and 4 females and 0.075 mg/kg bw, 4 males. Depression of erythrocyte cholinesterase activity (>20%) was seen at 0.05 and 0.075 mg/kg bw. Only one subject (0.075 mg/kg bw group, actual dose 0.06 mg/kg bw) developed symptoms which were reported to be related to aldicarb.

3.3 Assessment

The ADI for aldicarb was established on the basis of the no adverse effect level for inhibition of erythrocyte acetylcholinesterase activity in the human volunteer study. Using a safety factor of 10, this gives an ADI of 0.003 mg/kg bw. Since this human volunteer study was a single dose experiment it can be concluded that exactly the same no effect level and safety factor should be used to derive the acute reference dose.

The animal data on the toxicity of aldicarb and aldicarb metabolites indicates another area which ought to be considered in the acute dietary risk assessment for aldicarb. The sulphoxide and the sulphone are plant and animal metabolites of aldicarb, which are generally

included in the measured total residue of aldicarb. If residue data are available which can differentiate the sulphone from the other components of the residue then these data may enable further refinement of the risk assessment, since the sulphone is some 10-20 times less toxic than the parent aldicarb.

4 POTENTIAL HIGHEST INTAKES OF ALDICARB

4.1 Intake from potatoes

The highest potential intakes have been calculated for adults, children and infants for two different consumption patterns. These involve consumption of a single baked potato (which may have a residue at the highest level recorded for a single tuber) or consumption of a large portion of new potatoes (where one tuber could have a residue at the highest level recorded for a single tuber, while other tubers in the portion have residues at the MRL). This process could be considered to result in an over-estimate of aldicarb intake, since it is unlikely that a number of tibers would all have residues at the MRL. A more likely scenario would have one tuber containing a residue at the highest level recorded for a single tuber, while other tubers in the portion have residues at the mean residue recorded in the trials. No processing reduction factors have been included since it has been assumed that the maincrop potatoes may be microwave baked, and the new potatoes may be microwave boiled. However, if these intakes are to be compared with the acute reference dose for aldicarb, a 'reduction' factor could be allowed for the content of aldicarb sulphone in the terminal residue. If it is assumed that the residue consists of 80% parent and sulphoxide and 20% sulphone, then it may be possible to reduce the intakes to 80% of their original value. The resultant intakes are tabulated below:

Commodity/ Population subgroup	Residue (mg/kg)	Consumption (g)	Intake (mg)	Intake[(a)] (mg/kg bw)	Intake[(b)] (mg/kg bw)
Maincrop potatoes					
Adults (60 kg)	0.76	220	0.167	0.003	0.002
Children (30 kg)	0.76	180	0.137	0.005	0.004
Infants (15 kg)	0.76	100	0.076	0.005	0.004
New potatoes					
Adults (60 kg)	0.552	220	0.121	0.002	0.002
Children (30 kg)	0.565	175	0.099	0.003	0.002
Infants (15 kg)	0.587	120	0.070	0.005	0.004

(a): No allowance for sulphone content
(b): With allowance for sulphone content

4.2 Intake from bananas

The highest potential intakes have been calculated for adults, children and infants for one consumption pattern. This involves consumption of two large bananas at a single sitting. No change is made in this consumption figure for different population subgroups. Although this may seem very large for infants and children, this intake is considered to be possible. The caculation assumes a residue at the current MRL. Although no allowance is made for the possibility of residues in individual fruits being higher than the MRL, this is offfset by proposals to change agricultural practice in a manner intended to reduce residues such that an

MRL may be proposed at 0.1 mg/kg. In contrast with the situation for potatoes, there is no possibility of using a 'reduction' factor for any sulphone component in the terminal residue in bananas. The resultant intakes are tabulated below:

Population subgroup	Residue (mg/kg)	Consumption (g)	Intake (mg)	Intake (mg/kg bw)
Adults (60 kg)	0.3	240	0.8	0.013
Children (30 kg)	0.3	240	0.8	0.027
Infants (15 kg)	0.3	240	0.8	0.053

4.3 Intake from potatoes and bananas combined

Although it is considered feasible to assume that potatoes and bananas may be consumed at a single 'meal time' sitting, it is considered not appropriate to combine the overtly large portion sizes used in the calculations for the single commodities alone. Intakes have been combined for consumption of an 'average' portion of potatoes with one banana. The resultant intakes are tabulated below:

Population subgroup	Intake from potatoes (mg/kg bw)	Intake from bananas (mg/kg bw)	Combined intake (mg/kg bw)
Adults (60 kg)	0.0016	0.0065	0.0081
Children (30 kg)	0.0023	0.0135	0.0173
Infants (15 kg)	0.0039	0.0265	0.0304

5 RISK ASSESSMENT

It is evident that the highest potential intakes can exceed the acute reference dose, particularly in children and infants. It is also obvious that the potential intake from bananas far exceeds the intake from potatoes. The highest intake from bananas varies from 0.013 mg/kg bw (in adults) to 0.053 mg/kg bw (in infants). These intakes can be compared not only with the acute reference dose of 0.003 mg/kg bw, but also with the results of the human volunteer studies which indicate that although clinical signs of intoxication were clearly seen at doses of 0.1 and 0.26 mg/kg bw, only very minor signs were seen at 0.06 mg/kg bw, and in all cases the signs were reversible. It should also be noted that even if the MRL for bananas is reduced to 1 mg/kg and this figure were used in the intake calculations, the resultant potential intakes would still be in excess of the reference dose.

Since the individual occurence of these intakes is without adverse, lasting sequelae, the real significance of the intakes is therefore governed by the probability of these intakes actually occuring in real life. In this analysis data relating to food consumption and residue level have been represented by single numbers, erring on the side of caution (using large portion sizes and MRLs). It is known that these data points are continuous variables and information is available to characterise the distribution of residue and food intake data. Use of statistical modelling techniques should enable information to be obtained about the distribution of the resultant aldicarb residue intake.

REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF CYHEXATIN

(Opinion expressed by the SCP on 26 January 1993)

Background

The toxicology of cyhexatin has been evaluated by the JMPR during 1970-1985. In 1980 an ADI of 0.008 mg/kg b.w. was allocated. In 1988 cyhexatin of Dow Chemical was withdrawn from the market because of teratogenic effects in rabbits after oral and dermal exposure. Since there were other manufacturers of cyhexatin, which supplied additional data, the teratogenicity and reproduction aspects were evaluated by the JMPR in 1989 and 1991. On basis of one two-generation reproduction study with an NOAEL of 0.1 mg/kg b.w. the ADI was lowered to 0.001 mg/kg b.w. Since azocyclotin (which has not been found teratogenic at dose levels up to 1 mg/kg b.w.) gives cyhexatin as the main residue in crops the ADI of azocyclotin was also lowered to 0.001 mg/kg b.w.

The EC Commission has requested to review the relevant reproduction studies and comment on the recent JMPR conclusions.

Absorption, distribution and excretion

In rats blood levels of tin following oral administration of cyhexatin peaked in 3-4 hours and then declined almost to control values in 24 hours. An oral study in rats using technical and micronized cyhexatin resulted in peak blood levels of tin at 3 hours with technical material and 4 hours with micronized material. Levels with micronized material were higher than those with technical material. Dermal exposure of rabbits resulted in similar blood levels of tin with both technical and micronized material. Peak values in blood were found at 8 hours in both cases (WHO/JMPR, 1991).

In pregnant rabbits, receiving 3.0 mg technical cyhexatin kg/b.w/day orally on gestation days 6-18, peak maternal blood tin levels were achieved about 3 hours after dosing. Tin half-life in maternal blood was 8.17 hours. Tin levels in amniotic fluid, placentae, and pups were significantly increased on day 19. Tin levels in pup brains were also elevated. By day 26, tin levels in treated animals were comparable to those in controls, except in brain where levels were slightly elevated. On day 19 mean pup weights were comparable to controls, but they were reduced by day 26 (WHO/JMPR, 1991).

Studies on reproduction

Oral studies

Rat

In a two generation study groups of 30 Sprague-Dawley rats/sex were fed diets yielding dose levels of 0, 0.1, 0.5, or 6.0 mg cyhexatin (purity 95.6%)/kg b.w./day, for the duration of a two-generation study. In the first generation one litter (F_{1a}) and in the second generation two litters (F_{2a} and F_{2b}) were bred. Observations included clinical signs, mortality, body weight, food consumption, mating performance and reproductive parameters. Parents were observed for gross and histopathology. Pups were sexed, weighed and observed for gross abnormalities.

Body weight of F_0 parents was decreased at 6 mg/kg in males and at 0.5 and 6 mg/kg in females. In F_1 parents body weight gain was depressed at 6 mg/kg in both sexes. Food intake in F_0 was slightly decreased in both sexes at 6.0, in females at 0.5 and in F_1 males at 6 mg/kg. Gross pathology of F_0 and F_1 parents showed decreased abdominal fat and dark livers of both sexes at 6 mg/kg. Histopathology showed increased bile duct hyperplasia and periductular inflammation, as well as reduced hepatic glycogen in both sexes at 6.0 mg/kg. In the F_{1a}, F_{2a} and F_{2b} breeding pup weight gain was depressed at 6 mg/kg. In the the F_{2a} and F_{2b} breeding a slight decrease in pup survival was noted at 6 mg/kg. The NOAEL for this study appears to be 0.1 mg/kg b.w. with some indication of decreased F_0 female body weight gain at 0.5 mg/kg b.w. (It should be noted that no detailed individual data were available except those on histopathological examination) (Breslin et al, 1987).

A second two-generation study has been reported. In this study two generations were studied with one litter in the first generation and 2 litters in the second generation. The second of these litters (F^{2b}) was terminated prior to parturition and examined following ceasarian section. Groups of 25 OFA SD rats/sex/dose level were fed diets containing 0, 10, 30 or 100 ppm micronized cyhexatin (purity 96%)/kg b.w. All litters were culled to 8 (4/sex where possible) on day 4 post-partum.
Observations were made for clinical signs, mortality, body weight, food consumption, mating performance, reproductive parameters. Pups were sexed, weighed and observed for gross abnormalities.

Body weight gain of all F_0 females showed a dose related decrease (significant at 30 and 100 ppm). Weight gain of F_0 males was only decreased at 100 ppm. In the F_{1a} females and males body weight gain was reduced in all groups (significant at 30 and 100 ppm). Food consumption was reduced in F_0 and F_1 parents at 30 and 100 ppm. In the F_0-F_{1a} reproduction phase the number of females with live pups was reduced at 100 ppm as were gestation, viability and weaning indices. Total litter loss was found in 0, 2, 3 and 4 rats at 0, 10, 30 and 100 ppm. Survival to weaning was reduced in 30 and 100 ppm and pup weights at weaning also showed a dose related reduction at 30 and 100 ppm. Litter size and implantations/dam were reduced at 100 ppm. Pup eye opening was delayed by about 2 days in the 100 ppm group. In the F_1-F_{2a} breeding the pre-coital interval between pairing and mating was increased at 100 ppm, but was still within one

oestrus cycle. At 100 ppm litter size and implants/dam were reduced, incidence of stillbirths was slightly increased and pup survival to weaning was decreased. During lactation pup weight gain was markedly reduced at 100 ppm and slightly reduced at 30 ppm. Eye opening and incisor eruption were delayed at 30 and 100 ppm. At day 21 pupillary reflex was absent in 7 pups from 4 litters.

In the F_{2b} the number of corpora lutea was reduced at 100 ppm. The only malformation in this study occurred at 30 ppm (cleft palate and thoracic blood vessel effects). This is not considered as a substance related effect. Uterine dilation and torsion at 100 ppm were increased but were within historical control values. Skeletal variants showed an increased incidence of sternal ossification defects at 30 and 100 ppm but the incidence of asymmetric sternebrae was decreased. There was no evidence of an irreversible structural effect. The NOAEL in this study is considered to be 10 ppm, equivalent to 0.5 mg/kg b.w./day (Barrow, 1990).

Studies on teratology

Oral studies

Rat

Four groups of 30 pregnant Sprague-Dawley rats received day 6 through 15 of gestation by gavage 0, 0.5, 1.0 or 5.0 mg cyhexatin (purity 95.6%)/kg bw/day suspended in corn oil. On day 20 of gestation the animals were sacrificed.

Slight maternal toxicity was observed at 5.0 mg/kg. Microphtalmia was observed in 1, 2 and 2 (2) fetuses (litters) at 0.5, 1.0 and 5.0 mg/kg, respectively. Tail anomalies (short, short and thread-like or short and bent) were observed in 1, 0, 1 and 3 (3) fetuses (litters) at 0, 0.5, 1.0 and 5.0 mg/kg b.w., respectively. One fetuses at 5 mg/kg had fused skull bones. The occurrence of 25 or 27 pre sacral vertebrae was observed at 5 mg/kg in 6 (6) fetuses (litters). The NOAEL is considered to be 1.0 mg/kg b.w. based on fetotoxicity (Aldridge, et al 1986).

Rabbit

In a probe study groups of 7 pregnant NZW rabbits received day 6 through 18 of gestation by gavage 0, 5, 10 or 20 mg cyhexatin (purity not given)/kg b.w./day suspended in corn oil. During dosing period 2, 7 and 7 rabbits died at 5, 10 and 20 mg/kg, probably due to wrong dosing because lesions in the trachea and lungs were present. The surviving animals had stomach erosions or ulcers. Four of the 5 surviving rabbits at 5 mg/kg had resorptions (Berdasco et al, 1986).

In a second probe study groups of 7 rabbits of the same strain were dosed with 0, 1, 5 or 10 mg cyhexatin/kg in 0.5% aqueous Methocel on days 7 through 19 of gestation. Again mortality occurred, 2, 1 and 1 deaths at 1, 5 and 10 mg/kg, but there were no indications for mistubing. Stomach erosion/ulcers were observed in one animal at 5 mg/kg and in 4 at 10 mg/kg. Peritoneal blood was observed in 4 females at 10 mg/kg and was probably due to recent

abortions. Slight maternal toxicity (reduced body weight gain) was observed at 5 and 10 mg/kg. At 10 mg/kg 4 females totally resorbed and/or aborted. The remaining two females had resorptions and the number of viable fetuses was significantly reduced compared to controls. At 5 mg/kg there was an increased incidence of resorptions, with one litter totally resorbed, and a decreased number of viable fetuses (Berdasco, 1986).

As a result of the above probe studies a study was conducted on 4 groups of 20 pregnant rabbits of the same strain with dose levels of 0, 0.5, 1.0 and 3.0 mg cyhexatin (purity 95.5%)/kg b.w./day suspended in 0.5% aqueous Methocel on days 7 through 19 of gestation. On day 29 of gestation all rabbits were sacrificed.

One rabbit at 0.5 mg/kg and 4 rabbits at 3.0 mg/kg aborted. One rabbit each at 1.0 and at 3.0 mg/kg delivered spontaneously and one rabbit each at 0.5 and 3.0 mg/kg resorbed their litters. The number of viable fetuses per dam was reduced in the 3.0 mg/kg. The number of dams with two or more resorptions was increased. Post implantation loss was dose relatedly increased at 1.0 and 3.0 mg/kg b.w. Hydrocephalus was observed in 8 (4) fetuses (litters) at 3.0 mg/kg. One aborted fetus at 3.0 mg/kg had a dome-shaped head, but was not examined viscerally. The NOAEL in this study is considered to be 0.5 mg/kg (Schardein et al, 1986).

To confirm the results of the study above, another study was conducted with groups of 27 pregnant NZW rabbits with dose levels of 0, 0.75 or 3.0 mg cyhexatin (purity 94.8-95.5%)/kg b.w/day in 0.5% aqueous Methocel during days 7 through 19 of gestation. On day 28 of gestation of gestation the rabbits were sacrificed.

There were 2, 7 and 4 deaths at 0, 0.75 and 3.0 mg/kg, respectively. Most of these rabbits had lung or thoracic cavity lesions suggesting dosing problems. Abortions occurred in 3 rabbits at 0.75 mg/kg and in 12 rabbits at 3.0 mg/kg. In 5 of these hairballs were found in the stomach. At 3.0 mg/kg maternal toxicity was observed. There was an increased incidence of resorption and a decreased number of live fetuses in the 3.0 mg/kg group (according to the WHO/JMPR only 7 rabbits are remaining in this group instead of the expected 11). The fetuses were observed only for external and visceral examination and the total incidences of any malformation were 3 (3), 10 (7) and 11 (5) fetuses (litters) at 0, 0.75 and 3.0 mg/kg, respectively. The most common finding was hydrocephalus with incidences of 2 (2), 7 (5) and 9 (4) fetuses (litters), respectively. Dilated cerebral ventricles was observed in one fetus each at 0.75 and 3.0 mg/kg, in litters with no hydrocephalic fetuses. The NOAEL for maternal toxicity is considered to be 0.75 mg/kg b.w, however for teratogenicity (hydrocephalus) no NOAEL can be established (Kirk et al, 1987a).

Groups of 24 mated rabbits (Hybrid HY/Cr White New Zealand) were given by gavage 0, 3.0 (technical, 96%) or 3.0 (pure) mg cyhexatin/kg b.w. in 0.5% aqueous carboxymethyl cellulose, on days 6 through 18 of gestation. The surviving rabbits were sacrificed on day 29.

In the control and technical cyhexatin treated groups mortality occurred, but not with the pure material. In the technical cyhexatin group 3 rabbits aborted. The group given pure cyhexatin had slightly fewer viable fetuses as a result of fewer corpora lutea and implantations. In the technical group one fetus was observed with a dome-shaped head associated with dilated ventricles of the brain. One fetus given pure cyhexatin also had dilated ventricles of the brain. The

total incidence of major malformations was 1, 2, and 2 (2) fetuses (litters) at 0, 3.0 (technical) or 3.0 (pure) mg/kg (Monnot, 1989a)

In a second experiment 24 pregnant rabbits/group were treated with 0, 0.5, 0.75 or 1.0 mg (technical, 96%) mg/kg b.w. There were 1-3 deaths in each group of rabbits, but the incidence was not dose related. Abortions were observed in the control group and at 0.5 mg/kg, respectively 3 and 2 rabbits. In the 0.5 mg/kg group one fetus was found with a dome-shaped head associated with dilated ventricles. The incidence of major malformations was 1 (1), 3 (3), 4 (4) and 3 (3) fetuses (litters) at 0, 0.5, 0.75 and 1.0 mg/kg. Retinal detachment was observed in 2, 3 and 1 fetus at 0.5, 0.75 and 1.0 mg/kg (Monnot, 1989b).

In these latter two studies no apparent treatment related maternal toxicity and embryo/fetotoxicity, nor teratogenic effects were observed. The NOAEL was the highest dose tested, 3.0 mg/kg b.w. (Monnot, 1989a & b)

In a recently performed study groups of 14 to 17 pregnant rabbits (New Zealand White) received orally 0.75, 1.5 or 3 mg cyhexatin (purity 97%, manufactured in Kentucky)/kg b.w./day dissolved in 0.5% methyl cellulose mucilage, days 6-19 of gestation. Three other groups were treated similarly, but with cyhexatin of 98% purity (manufactured in the Netherlands). Two additional groups of 8 or 9 pregnant rabbits received received 3.0 mg cyhexatin (purity 99.7%)/kg b.w. dissolved in 0.5% methyl cellulose mucilage or in 1% Cremophor EL. The control group received 0.5% methyl-cellulose mucilage.

Particle size of the three samples was measured, with the following results.

	Particle size (microns)			Surface area
	%90[1]	%50[2]	%10[3]	(m^2/gm)
97%, Kentucky	315	161	8	0.34
98%, Netherlands	140	38	9	0.59
99.7%, High purity	80	27	7	0.60

[1] 90% of particles are less than this size
[2] Median particle size
[3] 10% of particles are less than this size

In the study in which Kentucky technical material was used 1/15 rabbits died at 1.5 mg/kg and 1/15 rabbits died at 3.0 mg/kg. Abortions occurred in 1/14 and 1/14 rabbits at 1.5 and 3.0 mg/kg. At 3.0 mg/kg weight gain and food intake were reduced during the treatment period. Incidences of fetal skeletal aberrations with a dose-related relationship included 13/13 ribs, or short 13 th rib at 3.0 mg/kg. Soft tissue examinations indicated an increased incidence of unilateral or bilateral folded retinas at all dose levels, exceeding historical control levels. The significance of this is uncertain. Incidence of slightly increased dilation of lateral 3rd ventricle of the brain was observed in 1/24 and 2/34 heads examined at 1.5 and 3.0 mg/kg, respectively. The

historical control range was exceeded only at 3.0 mg/kg b.w./day. Based on folded retinas no NOAEL could be established.

In the study in which The Netherlands technical was used 4/17 maternal deaths were found at 3.0 mg/kg. Abortions occurred at 1/16 and 2/17 rabbits at 0.75 and 3.0 mg/kg. A dose related decrease in bodyweight gain was observed at all dose levels during the treatment period. Food consumption was slightly decreased at 1.5 and markedly decreased at 3.0 mg/kg. Two out of the remaining 11 rabbits aborted at 3.0 mg/kg. These abortions followed marked maternal weight loss. A dose-related increased preimplantation loss occurred at all dose levels (12.9, 25.5, 27.3 and 28.7% at 0, 0.75, 1.5 and 3.0 mg/kg, respectively). Post-implantation losses are increased at 3.0 mg/kg (19.5%). Litter size was reduced at all dose levels. Incidences of fetal skeletal aberrations which showed a dose relationship included an increased incidence of 12/13 ribs and increased incidence of thickened ribs at 3.0 mg/kg. Soft tissue examinations indicated an increased incidence of unilateral or bilateral folded retinas at all dose levels, exceeding historical control levels. The significance of this finding is uncertain. Incidence of slightly increased dilation of lateral 3rd ventricle of the brain was observed in 1/36 heads examined at the 1.5 mg/kg group. This is within the historical control incidence. Based on preimplantation loss, reduced litter size and folded retinas no NOAEL could be established.

Maternal mortality occurred with the pure cyhexatin suspended in methyl cellulose mucilage (2/9), although no maternal deaths occurred in the Cremophor EL group. Abortions occurred with the pure cyhexatin in both methyl cellulose (2/9) and Cremophor EL (3/7). Maternal body weight was markedly reduced during treatment in both solvent groups. Food intake was markedly reduced, especially in the Cremophor EL group. Pre-implantation losses were increased in both solvents (39% for Methocel and 36% for Cremophor), due to extremely high levels in one individual in each group. Litter size was reduced in both groups. Soft tissue examinations indicated an increased incidence of unilateral or bilateral folded retinas with the Cremophor group. Incidence of slightly increased dilation of lateral 3rd ventricle of the brain was observed in 1/12 heads examined in the methocel group. The number of pups was insufficient for a valid examination of the skeletal variants (Bailey et al, 1990).

This study is difficult to evaluate because too many variables were studied in one experiment. It seems however that a higher purity, combined with a lower particle size, caused a higher maternal and fetal toxicity. The measured incidence of folded retinas, which was found in all tested groups is difficult to interpret. If this parameter is excluded the NOAEL's for maternal and fetotoxicity in the study with Kentucky and the Netherlands material are 1.5 and <0.75 mg/kg b.w., respectively.

Dermal studies

Rabbit

Groups of 16 pregnant New Zealand White rabbits were given dermal doses of 0, 0.5, 1.0 or 3.0 mg cyhexatin (purity 94.8-95.5%)/kg b.w. on days 7 through 19 of gestation. The test material was suspended in in 0.5% aqueous Methocel. The test material was applied to a clipped areas on

the back of each animal and covered with an occlusive bandage for a 6-hour period. The animals were sacrificed on day 28 of gestation.

There was a dose related increase in lesions at the site of application (erythema/eschar, edema and fissuring/scaling) which increased with dosing. At 3.0 mg/kg acanthosis, hyperkeratosis with slight inflammation were seen histologically. Hydrocephalus was observed in 4 (3) fetuses (litters) in the 3.0 mg/kg group, but not in any other group although dilated cerebral ventricles were seen in 3 fetuses from one control litter and one fetus in the 3.0 mg/kg group. Since hydrocephalus was also observed in fetuses of rabbits given cyhexatin orally the incidence of this lesion is considered to be treatment related. Retroesophageal subclavian was observed in one fetuse at 0.5 mg/kg and in one fetus at 3.0 mg/kg. Multiple facial anomalies (including anophthalmia) was observed in one fetus at 1.0 mg/kg b.w. and cleft palate and persistent truncus arteriosus with ventricular septal defect each in one fetus at 3.0 mg/kg. The cleft palate occurred in a fetus with hydrocephalus. The NOAEL for teratogenicity is considered to be 1.0 mg/kg b.w./day based on hydrocephalus (Kirk et al, 1987b).

In a similar study groups of 24 pregnant HY/Cr New Zealand White rabbits were given daily dermal doses of 0, 0.5, 1.0 or 3.0 mg cyhexatin (96% pure)/kg b.w. days 6-18 of gestation. The testmaterial was suspended in 0.5% aqueous carboxymethyl cellulose. The test material was applied to shaved areas on the backs of the rabbits so that test material was not applied more than twice on the same site without an interval of at least seven days. The test site was given a slight massage for about one minute after application of the test material. It is not indicated if the site was occluded or if any other precautions were taken to prevent oral ingestion. On gestation day 29 the females were sacrificed.

Erythema, atonia and desquamation were observed at the treatment site of all three dose levels. Cracking of the skin was observed in 6/24 rabbits at 1.0 mg/kg and in 11/24 rabbits at 3.0 mg/kg. Two females aborted, one at 0.5 mg/kg and one at 3.0 mg/kg. There was no dose-related increase in number of malformations. Arthrogyposis was observed in one fetus at 1.0 mg/kg and in two fetuses at 3.0 mg/kg.
There was no indication for teratogenicity. 3.0 mg/kg is considered to be the NOAEL (Monnot, 1989c).

Evaluation

The results of the teratogenicity studies in rabbbits have been summarized in table 1. From the studies supplied by Dow Chemical it appears that hydrocephaly was observed in all three studies. However, the JMPR concluded that these studies were of uncertain validity because of non-homogeneity of the test material and infections in the first study, possible infections in the second study and inhomogeneity and severe skin irritation effects in the third.
The studies supplied by Oxon did not show embryotoxic or teratogenic effects. In a study in which two different batches of cyhexatin were used a possible teratogenic effect was found with one batch at the highest dose level of 3 mg/kg b.w. The other batch showed high maternal and fetotoxicity at all dose levels (LOAEL 0.75 mg/kg b.w.). Apart from the Dow studies an NOAEL for teratogenicity can be put at 1.5 mg/kg b.w. or higher.

In the first reproduction study in rats maternal toxicity was observed at 6 and 0.5 mg/kg b.w. At 6 mg/kg b.w. pup weight gain and pup survival were lower. The NOAEL was 0.1 mg/kg b.w. In the second reproduction study maternal toxicty was observed at 30 and 100 ppm (equivalent to 1.5 and 5 mg/kg b.w.) Pup weight gain and pup survival were also decreased at these dose levels. In the last breeding the animals were studied for teratogenic and embryotoxic effects. Apart from skeletal variants at 30 and 100 ppm no effects were observed. The NOAEL is 10 ppm, equivalent to 0.5 mg/kg b.w.

In 1980 the JMPR allocated an ADI of 0.008 mg/kg b.w., based on the no effect level (0.75 mg/kg b.w.) in 2-year dog feeding studies evaluated in 1970. The receipt of the long-term study in rats (NOAEL 1 mg/kg b.w./day) and additional studies in dogs justified the alteration of the temporary ADI set in 1970 and extended in 1973 and 1978 to a full ADI. In 1990 the JMPR has based an ADI on the NOAEL of 0.1 mg/kg b.w. in the first reproduction study on rats. Since this level is much lower than the NOAEL for teratogenic effects (1.5 mg/kg b.w or higher) or fetotoxic effects (<0.75 mg/kg b.w.) this ADI of 0.001 mg/kg b.w can be accepted for the evaluation of the occurrence of residues of cyhexatin and azocyclotin in food.

TABLE 1. Toxicological evaluation of rabbit teratogenicity studies

	Maternal toxicity		Embryo/Foetal toxicity		Teratology		Reference
Doses(mg/kg)	NOAEL	Effect	NOAEL	Effect	NOAEL	Effect	
Oral rabbit							
0, 0.5, 1.0, 3.0	3.0	none	0.5	incr. post-implantation loss	1.0	hydrocephalus	Schardein, et al (1987) (Dow Chemical)
0, 0.75, 3	0.75	decr. body weight	<0.75	abortions	<0.75	hydrocephalus	Kirk et al, 1987a (Dow Chemical)
0, 3.0 (tech), 3.0 (pure)	3		3		3		Monnot, 1989a
0, 0.5, 0,75 1.0	1		1		1		Monnot, 1989b
0, 0.75, 1.5, 3.0 (Kentucky)	1.5	decr. body weight	1.5[1]	skeletal aberrations	1.5	dilation of lateral 3rd ventricle of the brain	Bailey, 1990
0, 0.75, 1.5, 3.0 (Netherlands)	<0.75	decr. body weight	<0.75	incr. pre-implantation loss	3		Bailey, 1990
Dermal rabbit							
0, 0.5, 1.0 3.0	<0.5	skin irritation	3		1	hydrocephalus	Kirk et al, 1987 (Dow Chemical)
0, 0.5, 1.0, 3.0	<0.5	skin irritation	3		3		Monnot, 1989c

[1] excluding increased incidence of unilateral or bilateral folded retinas

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF DIMETHOATE

(Opinion expressed by the SCP on 23 April 1994)

BACKGROUND AND TERMS OF REFERENCE

Dimethoate is a contact and systemic insecticide and acaricide which has approvals for use in all member states. The toxicity of dimethoate has been considered by the World Health Organisation JMPR in 1963, 1965, 1967, 1984 and 1987. In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the ADI for dimethoate, with particular reference to data generated recently, which may have altered the ADI since last consideration by the JMPR.

1 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

The JMPR evaluation of the FAO/WHO meeting in 1967 indicated that dimethoate is rapidly absorbed from the gut and radioactivity, using ^{32}P-labelled material, was concentrated in the liver, bile, kidneys and the urine. There was no accumulation in fat tissue. In rat and man up to 90 % of radioactivity was found in the urine after 24 hours; in guinea pigs 25 to 40 % is excreted in the faeces. One of the papers reviewed indicated that up to 18% of administered activity was excreted in the expired air. Four metabolites with anti- cholinesterase activity have been identified in the rat and man. One seems to result from thiono oxidation leading to the formation of the oxygen homologue of dimethoate, i.e. omethoate; this step was followed by hydrolysis to a thiocarboxyl product, said to be the main metabolite in rats and man (Ref. 1). A published paper by Hassan *et al.* appeared to confirm these indications (Ref. 2).

2 TOXICITY

2.1 ACUTE TOXICITY, IRRITATION AND SENSITISATION

The acute oral toxicity of dimethoate gave LD50's of approximately 310 mg/kg in rats, 150 mg/kg in mice and 55 mg/kg in hens. Signs of toxicity were those typical of cholinesterase inhibition; there was no evidence of delayed neurotoxicity in hens. In most acute studies in which signs of reaction were seen there were minor reductions in body weight gain and food consumption; this deficit was made up in the second week of the observation period. The acute toxicity of dimethoate formulations did not differ significantly from that of the technical material, when considered in terms of the active ingredient content. Undiluted dimethoate formulations were shown to be markedly irritant to the eye. Skin irritation was minimal and confined to slight, transient erythema. Dimethoate technical was not a skin sensitiser in guinea pigs in a Buehler type test, but when a 32.7% emulsifiable concentrate formulation was tested in a similar experiment, a positive sensitisation reaction was seen in one out of ten

guinea pigs. In a published German paper, dimethoate was cited in four human cases of contact dermatitis and sensitisation was confirmed in these individuals by patch testing (Ref. 3).

2.2 SUBACUTE/CHRONIC TOXICITY

In subacute and chronic studies the potential toxicity expressed through administration of dimethoate was limited by onset of cholinesterase inhibition. In studies in rats at dietary concentrations of 75 ppm or above, there were minor reductions in body weight gain and food consumption. Except in respect of cholinesterase activity, dimethoate did not affect the composition of the blood or urine. The liver weights of animals treated at the higher dosages or dietary concentrations, tended to be smaller than those of the control groups; there was, however, no microscopic correlation with this and it is unlikely to be of toxicological significance. The no effect levels were generally based on reductions, relative to controls, of cholinesterase activity in the brain or erythrocytes. Although several studies did not have no effect levels it was possible to identify doses at which no adverse effects were seen. On the basis of minimal reductions in cholinesterase activity in the brain or erythrocytes of between 10 and 20 %, the no adverse effect level in dogs was approximately 0.2 mg/kg bw/day. In rats the no adverse effect level over a life span study was 5 ppm, equivalent to approximately 0.25 mg/kg bw/day (Refs. 3, 4 and 5).

2.3 CARCINOGENICITY

Based on the results of rat and mouse bioassays conducted by the NCI and reported in 1977, and on the results of further studies in rats and mice reported in 1986, it was concluded that dimethoate is not carcinogenic in rodents (Refs. 3, 4 and 5). A review by Reuber (1984) suggested that dimethoate is carcinogenic, however this was not substantiated by any data, and it was concluded that the Reuber paper could be disregarded (Ref. 6).

2.4 MUTAGENICITY

Dimethoate was considered non-mutagenic in an Ames test, and an HGPRT assay *in vitro* gave no clear evidence of mutagenicity. An *in vivo* micronucleus assay in mice was negative, as was a dominant lethal assay in mice and an *in vivo* chromosome aberration study in mice. Dimethoate was considered positive in two *in vitro* UDS assays (using different methods of assessing uptake of tritiated thymidine into DNA), but negative in an *in vivo/in vitro* UDS assay. This latter study indicated an unusual S-phase activity and it is possible that this effect could have had a confounding effect in the *in vitro* studies. A review of the literature relating to the mutagenic potential of dimethoate revealed a number of positive results, notably in a salmonella assay using strain TA 100 and in a sister chromatid exchange study in mammalian cells *in vitro*. It was concluded that although *in vitro* studies indicate that dimethoate has mutagenic potential, *in vivo* assays indicate that this potential is not expressed *in vivo* (Refs. 3, 4 and 5).

2.5 REPRODUCTIVE TOXICITY

2.5.1 TERATOGENICITY

The teratogenic potential of dimethoate was investigated in rats, at dose up to 18 mg/kg bw/day, and rabbits, at doses up to 40 mg/kg bw/day. Neither species gave any evidence of teratogenic effect or fetotoxicity, though maternal toxicity was observed. The no effect level

for maternal toxicity was 10 mg/kg bw/day in rabbits and 6 mg/kg bw/day in rats (Refs. 3 and 5).

2.5.2 MULTIGENERATION STUDIES

In a recent multigeneration study in rats, which has never been reviewed in a JMPR evaluation, groups of 28 male and 28 female Sprague Dawley rats received dimethoate by dietary administration at levels of 0 (Control), 1, 15 and 65 ppm. Treatment was continued for 10 weeks prior to mating and then throughout mating, gestation and lactation for at least two matings in each of 2 generations. Treatment at 65 ppm was associated, in the parent animals, with marked reductions in plasma, erythrocyte and brain cholinesterase activity in both generations, slightly reduced body weight gain, increased food intake and reduced water intake, and a reduction in the number of successful matings, along with an associated increase in the number of apparently infertile males and females. In the offspring, at 65 ppm, a marked reduction in brain cholinesterase activity was seen, along with lower litter size at birth, increased pup mortality and slower pup growth. Similar effects were seen at 15 ppm, although to a much lesser extent, but it is notable that litter size, pup mortality and pup growth were still adversely affected at this dose. Treatment at 1 ppm (equivalent to approximately 0.08 mg/kg bw/day) was without adverse effects on adults or offspring (Ref. 7).

In an inadequate mouse multigeneration study conducted in 1965, there was no overt effect on reproductive capacity at dietary levels up to 50 ppm, even in the presence of cholinergic toxicity (Ref. 4).

2.6 OBSERVATIONS IN MAN

The JMPR documents reviewed indicated a no adverse effect level of 0.2 mg/kg bw/day in humans given that dosage for 39 days (Refs. 1, 4 and 5).

3 OVERVIEW AND ESTIMATION OF ADI

The World Health Organisation most recently evaluated dimethoate for acceptable daily intake in 1984 and 1987, using much of the data available for this review. In 1984 it was concluded that a temporary ADI of 0.002 mg/kg would be appropriate since the data base was incomplete. In 1987, when the data base was considered complete, an ADI of 0.01 mg/kg bw was established, based on the no effect level in humans (0.2 mg/kg bw/day), with a safety factor of 20. With the addition of the most recent data from the multigeneration study, in which an NOAEL of 0.08 mg/kg bw/day was derived, the maintenance of the ADI at 0.01 mg/kg bw would give a safety factor of only 8. A conventional safety factor of 100 is considered necessary and the ADI would therefore be 0.0008 mg/kg bw. It is considered not appropriate to base the ADI on the results of the human volunteer study, since the crucial end point (reproductive performance) was not assessed in the study in man.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF VINCLOZOLIN

(Opinion expressed by the SCP on 23 April 1994)

BACKGROUND AND TERMS OF REFERENCE

Vinclozolin is a protectant 3',5'-dichloroanilide fungicide used to control many diseases on a wide range of horticultural and agricultural crops. In the context of its work to establish maximum pesticide residue levels pursuant to the relevant Community legislation, the Commission requested the Scientific Committee for Pesticides to examine toxicological data relating to vinclozolin and to estimate its acceptable daily intake.

Since vinclozolin is structurally similar to procymidone, which has been reported to possess anti-androgenic potential, the data to be reviewed concentrated on studies likely to reveal any anti-androgenic effects (subchronic experiments in dogs and reproductive toxicology studies).

The toxicity of vinclozolin has previously been reviewed by the JMPR in 1986 and 1988, when an ADI of 0.07 mg/kg bw was estimated.

1. TOXICOLOGICAL ASPECTS

1.1. JMPR ADI

In 1986 the JMPR evaluated vinclozolin for the first time. Although the data package was considered incomplete, sufficient information was provided to estimate a temporary ADI. It was noted that a plant metabolite (Metabolite T) had been identified, which was not found in rats. It was concluded that vinclozolin had a low order of acute toxicity, that carcinogenicity studies demonstrated no potential for oncogenicity and that no specific effects were noted in mutagenicity, teratogenicity or reproduction studies. The temporary ADI was estimated as 0.04 mg/kg bw using the no effect level of 7 mg/kg bw/day based on histological changes in spleen, prostate and bone marrow in a six month dog study and a 200 fold safety factor. (Ref. 1)

In 1988 the JMPR evaluated limited toxicity data on metabolite T (acute toxicity and mutagenicity) and noted that the chemical was a transient residue in only two commodities. A full ADI was estimated as 0.07 mg/kg bw, using the same no effect level as that used in 1986, and a safety factor of 100. (Ref. 2)

1.2. Further toxicity study in dogs

In a study reported in 1986 (but not evaluated by the JMPR in 1986 or 1988), beagle dogs (6/sex/dose) were fed diets containing 0, 35, 75, 150 or 1500 ppm vinclozolin for 12 months. There were no deaths and no clinical signs of toxicity. Food consumption and body weight gains were not significantly affected by treatment. Haematological, clinical chemistry and urinalysis investigations were conducted predosing and at weeks 13, 26 and 52. At 1500 ppm, reticulocyte counts were increased in both sexes. Platelet counts were increased in males at 1500 ppm. Mean cell volume was increased in females at 1500 ppm. Total bilirubin concentrations were increased in both sexes at 1500 ppm. Pathology revealed a number of treatment-related changes, some of which were indicative of an anti-androgenic effect. Adrenal weights were markedly increased at 1500 ppm, and were slightly increased at 150 ppm in both sexes. The adrenals appeared enlarged in all males and in 5/6 females at 1500 ppm. The mean width of the adrenal cortex was increased in both sexes at 1500 ppm and was slightly increased in females at the lower dose levels. However, at these dose levels a dose-response relationship was not observed. Histopathology revealed progressive transformation and increased lipid content of the adrenals in all males and 5/6 females at 1500 ppm. Testes weights were increased in a dose-related fashion. At 1500 ppm, testes weights were markedly increased and at 150 ppm relative weights were significantly increased. At 1500 ppm, diffuse hyperplasia of the Leydig cells was observed in 5/6 males. Prostates of 1/6 and 2/6 males at 150 and 1500 ppm, respectively were reduced in size macroscopically. Histopathology revealed that 2/6 males at 150 ppm had slightly or moderately atrophied prostates and 5/6 males at 1500 ppm had slightly (1/6) or severely (4/6) atrophied prostates. This effect was also seen to a minimal extent in 1/6 males at 0, 35 and 75 ppm. Liver weights were increased in a non-dose-related fashion at up to 150 ppm and were markedly increased at 1500 ppm in males. Increased haemosiderin deposition was observed in livers of males at 1500 ppm and females at 150 and 1500 ppm. Spleen and thyroid weights were increased in males at 1500 ppm. Spleen weights were also slightly increased in females at 1500 ppm. The no adverse effect level was 75 ppm, equal to 2.4 mg/kg bw/day based on treatment-related pathological effects indicative of anti-androgenicity observed at 150 and 1500 ppm. (Ref. 3)

1.3. Reproductive toxicity

1.3.1. Teratology studies

1.3.1.1. Mouse

In a study reported in 1974, NMRI female albino mice were fed diets containing vinclozolin from day 1 to 19 of gestation in two different tests. In the first test, females received 0 or 60000 ppm vinclozolin, and the numbers of mice used were 24 and 28 respectively. In the second test, females received 0, 600 or 6000 ppm vinclozolin, and the numbers of mice used were 30 in each case. Females were killed on day 19 of gestation. Females treated at 6000 ppm did not increase in weight during gestation and had a decreased food consumption in the first six days of treatment. Females treated at 60000 ppm lost weight and had a markedly decreased food consumption. One female treated at 6000 ppm died on day 11, and all females treated at 60000 ppm died within the first nine days. Clinical signs of toxicity were observed at 60000 ppm only, and included ruffled coats and, prior to death,

apathy and signs of pronounced diuresis. Gross pathology revealed severe emaciation, slight atrophy of musculature and considerable loss in perirenal fatty tissue in animals which died. No implantation sites were detected in any female at 6000 or 60000 ppm, and hence no fetuses were observed at these dose levels. At the only dose available for studying effects on fetuses. 600 ppm. there were no treatment-related adverse effects observed. The no adverse effect levels for maternal toxicity and fetotoxicity were 600 ppm, equivalent to approximately 90 mg/kg bw/day. Teratogenicity was not observed at 600 ppm. (Ref. 4)

1.2.1.2. Rabbit

a) Study 1

In a study reported in 1980, New Zealand White female rabbits (15/dose level) were orally dosed at 0, 20, 80 or 300 mg/kg bw/day from day 6 to 18 of gestation. The animals were killed on day 29. There were no signs of toxicity or deaths which were obviously attributable to treatment. Body weight gain was not affected by treatment. There were no treatment-related effects on numbers of live young, sex ratio, embryonic deaths, pre-implantation losses, litter weights or fetal weights. Post-implantation losses were slightly increased non-dose-dependently in treatment groups, but within historical control values. There were no treatment-related major malformations or visceral anomalies. However, there was a slight non-dose-dependent increase in minor skeletal anomalies, but these were within historical control values. The no effect levels for maternal toxicity and fetotoxicity were >300 mg/kg bw/day. There was no evidence of teratogenicity. (Ref. 5)

b) Study 2

In a study reported in 1989, Himalayan female rabbits (15/dose level) were orally dosed at 0, 50, 200 or 800 mg/kg bw/day from day 7 to 28 of gestation. The animals were killed on day 29. At 200 mg/kg bw/day, 1 female aborted on day 26 and was killed. At 800 mg/kg bw/day, 1 female died. 1 female aborted and died, and 11 females aborted and were killed. Abortions occurred between days 21 and 27. Clinical signs of toxicity included reddish/brown discoloration of the urine in 13/15 females and apathy, hunched posture, conjunctivitis and urine-smeared fur in 1 to 2 animals at 800 mg/kg bw/day. At 200 mg/kg bw/day, the female which aborted displayed vaginal haemorrhage, and discoloration of the urine. A second female at this dose showed vaginal haemorrhage on day 24. Food consumption at 800 mg/kg bw/day was significantly reduced during the treatment period. At 200 mg/kg bw/day, food consumption was reduced, mainly from days 7 to 19. At 50 and 200 mg/kg bw/day, there were no treatment-related increases in pre-implantation and post-implantation losses. The only dam at 800 mg/kg bw/day with viable fetuses (4) had 3 post-implantation losses, as early resorptions. There were no treatment-related resorptions at 50 or 200 mg/kg bw/day. Sex ratios, numbers of live fetuses, placental weights and fetal weights were not affected by treatment at 50 or 200 mg/kg bw/day. There were no treatment-related external malformations or visceral abnormalities. At 800 mg/kg bw/day, 1/4 viable fetuses displayed malformations of the sternebrae. The relevance of this finding is questionable due to the small numbers of fetuses available for examination at 800 mg/kg bw/day. At 50 mg/kg bw/day, 1/94 fetuses displayed malformations of the sternebrae, but these were also present in the historical control data in the range 0 to 1% (mean 0.2%). No treatment-related changes to the male fetal genital organs were observed. The no effect

level for maternal toxicity was 50 mg/kg bw/day, based on reduced food consumption, abortion and clinical signs of toxicity at 200 mg/kg bw/day. The no effect level for embryotoxicity/fetotoxicity was 200 mg/kg bw/day, based on 3/7 resorptions as a consequence of maternal toxicity in the female alive at termination in the high dose group. There was no evidence of teratogenicity at 50 or 200 mg/kg bw/day. (Ref. 6)

c) Study 3

In a supplementary study reported in 1989, Himalayan female rabbits (20/dose level) were orally dosed at 0 or 400 mg/kg bw/day from day 7 to 28 of gestation. The animals were killed on day 29. At 400 mg/kg bw/day, 1 female aborted and died on day 19 and 9 females aborted and were killed between days 20 and 27. Clinical signs of toxicity included blood in the bedding of 2 treated females, one of which aborted. Of the treated group, 8/20 had red/brown discoloured urine. Food consumption was reduced at 400 mg/kg bw/day between days 7 and 26. Body weight gain was impaired in the same dose group between days 7 and 25. Pre- and post-implantation losses were increased in the treatment group, and as a consequence of the latter early resorptions were also increased. Numbers of live fetuses per litter were slightly decreased at 400 mg/kg bw/day. The ratio of males: females was decreased at 400 mg/kg bw/day (1 to 1.16 in the controls and 1 to 1.8 at 400 mg/kg bw/day). However, no treatment-related changes to the male fetal genital organs were observed. Fetal weights were increased in the treatment group, probably as a consequence of slightly decreased litter sizes. There were no external or soft tissue malformations at 400 mg/kg bw/day. Separated origins of the carotids was increased in the treatment group (10% in controls and 36% at 400 mg/kg bw/day). Incidences at 400 mg/kg bw/day were slightly higher than for the historical control data (mean = 19% and range 10 to 31%). There were no skeletal malformations in either group, and no treatment-related skeletal variations or retardations. No malformed sternebrae were observed at 400 mg/kg bw/day, which indicated that in the previous study, this effect was not treatment-related at 50 mg/kg bw/day. The no effect levels for maternal toxicity and embryotoxicity/fetotoxicity were <400 mg/kg bw/day. There was no clear evidence of teratogenicity at 400 mg/kg bw/day. However, the increased incidence of separated origins of the carotids at 400 mg/kg bw/day indicated a potential for teratogenicity at this severely maternally toxic dose level. (Ref. 7)

1.2.1.3. Rat

a) Study 1

In a study reported in 1987, female Wistar rats (25/dose level) were orally dosed at 0, 15, 50 or 150 mg/kg bw/day from day 6 to 19 of gestation. The animals were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions and no treatment-related effects on food consumption and body weights. Haematological investigations performed at day 20 revealed no treatment-related findings. At gross necropsy, no adverse findings were noted. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, fetal weights or placental weights. The anogenital distances were decreased in male fetuses at 150, and to a lesser extent at 50 mg/kg bw/day. The reductions in anogenital distances in males were considered in the study report to be indicative of the beginning of feminization of male fetuses, and may be caused by a possible hormonal (anti-androgenic) action on sexual differentiation. There were no other treatment-

related effects on male or female fetuses. No maternal toxicity was observed, and hence the no effect level was >150 mg/kg bw/day. No embryotoxicity or fetotoxicity were observed, and hence the no effect level was >150 mg/kg bw/day. The no effect level for teratogenicity was 15 mg/kg bw/day, based on reductions in anogenital distances at 50 and 150 mg/kg bw/day. (Ref. 8)

b) Study 2

In a study reported in early 1988, female Wistar rats (25/dose level) were orally dosed at 0, 50, 100 or 200 mg/kg bw/day from day 6 to 19 of gestation. The animals were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions and no treatment-related effects on food consumption and body weights. Haematological investigations performed at day 20 revealed no treatment-related findings. At necropsy, no treatment-related adverse signs were noted. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, placental weights or fetal weights. The only possible treatment-related morphological effect observed in the fetuses was a significantly increased incidence of symmetrically dumbbell-shaped thoracic vertebral bodies at 200 mg/kg bw/day. Such effects are indicative of retarded development. A slight, but not statistically significant, increase was observed at 100 mg/kg bw/day. Similar effects were observed in a follow-up study later in 1988. The no effect level for maternal toxicity was >200 mg/kg bw/day. The no effect level for fetotoxicity was 100 mg/kg bw/day, based on the possible treatment-related increase in symmetrically dumbbell-shaped thoracic vertebral bodies at 200 mg/kg bw/day. A no effect level for teratogenicity could not be established as no measurements of the anogenital distance of fetuses were performed. (Ref. 9)

c) Study 3

In a study reported later in 1988, female Wistar rats (25/dose level) were orally dosed at 0, 200 or 400 mg/kg bw/day from day 6 to 19 of gestation. The animals were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions and no treatment-related effects on food consumption and body weights. Haematological investigations performed at day 20 revealed no treatment-related findings. At gross necropsy, no treatment-related adverse signs were noted. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, fetal and placental weights or sex ratio. The anogenital distances were decreased in male fetuses in a dose-related fashion at both 200 and 400 mg/kg bw/day. These reductions in anogenital distances were indicative of the beginning of feminization of male fetuses, as reported previously. A treatment-related increase in dilated renal pelvis and hydroureter was also observed in fetuses of both sexes. Increases were statistically significant in fetuses at 400 mg/kg bw/day. These findings are considered to be indicative of a transient developmental delay. There was also a significant increase in numbers of fetuses with symmetrical dumbbell shaped thoracic vertebral bodies at 200 and 400 mg/kg bw/day. This was also seen in a previous study, and is indicative of retarded development. At 400 mg/kg bw/day, numbers of fetuses with accessory 14th ribs were also significantly increased. The no effect level for maternal toxicity was >400 mg/kg bw/day. The no effect level for fetotoxicity was <200 mg/kg bw/day, based on the increased incidences of symmetrical dumbbell shaped thoracic vertebral bodies at 200 and 400 mg/kg bw/day in both sexes. The no effect level for

teratogenicity was <200 mg/kg bw/day, based on the reductions in anogenital distance in male fetuses at 200 and 400 mg/kg bw/day. (Ref. 10)

d) Study 4

In another study reported in 1988, female Wistar rats (10/dose level) were orally dosed at 0, 600 or 1000 mg/kg bw/day from day 6 to 19 of gestation. The animals were killed on day 20. There were no deaths and no abortions. Clinical signs of toxicity included unsteady gait in 1 female at 600 mg/kg bw/day and 7 females at 1000 mg/kg bw/day, and piloerection in 2 females at 1000 mg/kg bw/day during the study. Food consumption was reduced at 1000 mg/kg bw/day from days 6 to 13. During the treatment period, water consumption was increased at 600 and 1000 mg/kg bw/day. Body weight gain of females at 1000 mg/kg bw/day was slightly impaired from day 8 to 10. Haematological investigations performed at day 20 revealed no treatment-related findings. Liver and adrenal weights were increased in a dose-related fashion. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, or placental weights. Fetal weights were decreased at 1000 mg/kg bw/day. When the sex ratio was determined by measuring the anogenital distance, 68 and 99% of fetuses at 600 and 1000 mg/kg bw/day were deemed to be female. However, internal examination of the fetuses showed that 39 and 52% were female fetuses at 600 and 1000 mg/kg bw/day. The anogenital distances were decreased in treated male fetuses. At 1000 mg/kg bw/day, incidences of symmetrical dumbbell shaped thoracic vertebral bodies were increased in fetuses. The no effect level for maternal toxicity was <600 mg/kg bw/day, based on increased liver and adrenal weights and necropsy findings at 600 and 1000 mg/kg bw/day. The no effect level for fetotoxicity was <600 mg/kg bw/day, based on increased incidences of hydroureter at 600 and 1000 mg/kg bw/day. The no effect level for teratogenicity was <600 mg/kg bw/day, based on reductions in anogenital distance in male fetuses at 600 and 1000 mg/kg bw/day. (Ref. 11)

e) Study 5

In a range finding study reported in 1989, female Wistar rats (10/dose level) were dermally administered 0, 300, 900 or 2500 mg/kg bw/day for 6 hours/day from day 6 to 19 of gestation. The test substance was applied on the dorsal area of the trunk under an occlusive dressing and the animals were killed on day 20. In females at all dose levels, adrenal and liver weights were increased, but not in a dose-related fashion. In male fetuses, anogenital distances were reduced but not dose-dependently. Incidences of dilated renal pelvis in fetuses were increased in a dose-related fashion in all dose levels, with statistical significance at 900 and 2500 mg/kg bw/day. The absence of dose relationship in some of the effects observed in this study was probably attributable to the limited dermal absorption of the rather pasty test substance suspension at 2500 mg/kg bw/day. (Ref. 12)

In the main study reported in 1989, female Wistar rats (15/dose level) were dermally administered 0, 60, 180 or 360 mg/kg bw/day for 6 hours/day from day 6 to 19 of gestation. The test substance was applied on the dorsal area of the trunk under an occlusive dressing and the animals were killed on day 20. In females, there were no deaths, no abortions and no signs of toxicity. There were no treatment-related effects on body weights or food consumption. Haematological investigations performed at day 20 revealed no treatment-related findings. Absolute liver weights were slightly increased at 180 mg/kg bw/day and significantly increased at 360 mg/kg bw/day. Absolute adrenal weights were significantly increased at 180 and 360 mg/kg bw/day but not dose-dependently. There were no treatment-related macroscopic pathological findings. There were no treatment-related effects on implantation losses, resorptions, numbers of fetuses, sex ratio, weights of fetuses or weights of placentae. The anogenital distances were decreased in males at 180 and 360 mg/kg bw/day. There were no other treatment-related malformations, variations or retardations in treated fetuses. The no effect level for maternal toxicity was 60 mg/kg bw/day, based on increased absolute adrenal and liver weights at 180 and 360 mg/kg bw/day. The no adverse effect level for fetotoxicity was >360 mg/kg bw/day. The no effect level for teratogenicity was 60 mg/kg bw/day, based on reductions in anogenital distances in male fetuses at 180 and 360 mg/kg bw/day. (Ref 13)

1.3.2. Multigeneration studies

a) Study 1

From 1975 to 1977, a 3 generation study was performed using Sprague Dawley rats (20/sex/dose level) fed diets containing 0, 162, 486 or 1458 ppm. There were 2 litters per generation, and the second litters were used as parent animals (F0, F1 and F2). The pre-mating dosing period was approximately 8 weeks for F0 males and 16 weeks for F0 females, 15 weeks for F1 and F2 males and 24 weeks for F1 and F2 females (including lactation). In pups and parents, there were no treatment-related deaths or clinical signs of toxicity. There were no treatment-related effects on food consumption and body weights. There were no treatment-related effects on litter size, malformations, birth weight of pups, sex ratio and behaviour. In parents, there were no treatment-related effects on fertility, pregnancy rate, duration of pregnancy, lactation or viability. Auditory acuity and ophthalmoscopic findings were not affected by treatment. At necropsy, there were no treatment-related effects on organ weights or at gross or microscopic examination. The no effect level was >1458 ppm, equivalent to approximately 73 mg/kg bw/day, since no treatment-related effects (including effects on general reproductive performance) were observed in the study. The results of this study are not in accordance with more recent work. However, the present study did not include investigations of anogenital distance in males, and diets were not analysed for the test substance, vinclozolin. (Ref 14)

b) Study 2

In a second multigeneration study, Wistar F0 parent rats (24/sex/dose) were fed diets containing 0, 50, 300, 1000 or 3000 ppm vinclozolin. Following a pre-mating period of 10 weeks, F0 animals were mated, twice, to produce F1a and F1b pups. F1a animals were used as F1 parents to produce F2a and F2b animals. Some F1b rats were raised to adulthood to observe development of sexual organs (FX animals). Similarly some F2a and

F2b rats were also raised to adulthood (FY and FZ respectively). The results of the study may be summarised in three sections: those findings indicative of general toxicity, effects on reproductive performance and signs of developmental toxicity, as tabulated below:

a) General toxicity

Dietary level (ppm)		Finding
50	-	
300	F0	Increased relative liver weight, marginal signs of anaemia (females), lenticular degeneration
	F1	As F0, plus increased adrenal weight, Leydig cell hyperplasia
	F2	As F1
1000	F0	Reduced food intake and weight gain, anaemia (females), increased liver, adrenal and testes weights, lenticular degeneration, hepatic single cell necrosis, Leydig cell hyperplasia
	F1	As F0, plus vacuolation of pituitary cells, lipidosis and hypertrophy of adrenal cells
3000	F0	Reduced food intake and weight gain, lenticular degeneration, anaemia (females), increased liver, adrenal and testes weights, hepatic single cell necrosis, lipidosis and hyperplasia of adrenal cells, vacuolation of pituitary cells, Leydig cell hyperplasia
	F1	As F0, plus benign Leydig cell tumours in some animals.

b) Effects on reproductive performance

Dietary level (ppm)		Finding
50	-	
300	F0	-
	F1	Possible reduction in fertility in males (all males eventually proved fertile)
1000	F0	-
	F1	Infertility of all males due to feminization of outer genital organs
3000	F0	Increased total litter loss, decreased number of delivered pups, infertility in males
	F1	Infertility of all males (feminization), infertility of 6 females

c) Signs of developmental toxicity

Dietary level (ppm)		Finding
50	F1	-
	F2	Reduced epididymides weight (no morphological changes)
300	F1	Slight functional reduction of prostate and coagulating gland, reduced epididymides weight
	F2	As F1, plus slight interstitial cell hyperplasia in testes and ovaries, some pups with reduced morphological development (delayed eye opening and pinna unfolding)
1000	F1	Decreased pup survival, feminization of males, reduced body weight gain, slightly reduced morphological pup development, reduced size and function of secondary male genital organs, atrophy of seminiferous tubules, interstitial cell hyperplasia in testes and ovaries
3000	F1	Increased number of still-born pups, decreased pup survival, feminization of males, reduced body weight gain, retarded morphological development, atrophy of primary and secondary male genital organs, interstitial cell hyperplasia in testes and ovaries.

In conclusion, it was shown that all F1 males and females at 300 ppm were fertile either at the F2B mating or by additional matings performed thereafter for those animals which could not prove their fertility in at least one of the scheduled matings for the F2A/F2B litters. The registrants therefore consider that "clear adverse effects on fertility were noted only at 1000 and 3000 ppm, whereas 300 and 50 ppm were without any adverse effects on male and female fertility in both parental generations". However, it must be noted that rats are particularly fertile animals and effects observed particularly at 300 ppm may be indicative of sub-fertility.

A dietary level of 50 ppm (equivalent to approximately 4.5 mg/kg bw/day) is considered to be a marginal effect level based on a possible treatment-related reduction in fertility of F1 males at 300 ppm, signs of delayed development at 300 ppm (reduction in numbers of pups with pinnae unfolding and eyes opening at the expected time) and reduced epididymal weight in F2 animals at 50 ppm. (Ref 15)

Toxicological overview and estimation of ADI

In the toxicity studies, vinclozolin and/or its metabolites caused toxic effects which were indicative of anti-androgenic activity. Anti-androgens are substances which prevent androgens from expressing their activity at target sites.

In reproductive toxicity studies the no adverse effect levels in teratology studies were substantially higher than those seen in the multigeneration studies. The crucial dose level to use for establishing an ADI could be considered to be 50 ppm (4.5 mg/kg bw/day), based

on a possible treatment-related reduction in fertility at 300 ppm in the 2 generation reproductive toxicity study. A larger than normal safety factor is considered necessary because this dose is not a clear no effect level and the effects seen in the study at high doses (abolition of reproductive capacity) are considered severe. Furthermore the mechanism of action of vinclozolin has not yet been clearly established, and the results of the teratology and multigeneration studies with vinclozolin suggest that the crucial dosing period for the expression of the anti-androgenic potential of vinclozolin could be quite early in pregnancy, at a time when many women may be unaware that they are pregnant. A safety factor of 500 is considered necessary.

A further multigeneration study in rats, long term studies in rats and mice, and investigative studies relating to the mechanism of toxicity of vinclozolin are known to be ongoing. The Scientific Committee for Pesticides has already reviewed preliminary results from some of these experiments and will review these formally when complete reports are available.

The no effect level in the 12-month dog study was equivalent to approximately 2 mg/kg bw/day, based on pathological changes indicative of anti-androgenicity. Although a safety factor of 100 could be considered appropriate for these effects, currently the multigeneration study is considered more crucial in determination of the ADI in view of the higher safety factor required. A temporary ADI of 0.01 mg/kg bw may therefore be derived, pending review of results of the ongoing studies.

The committee drew attention to the fact that the JMPR ADI for vinclozolin is 0.07 mg/kg bw, but noted that the JMPR has not reviewed many of the studies included in the present document. Members requested that the commission draw the attention of the JMPR to the conclusions of this current review.

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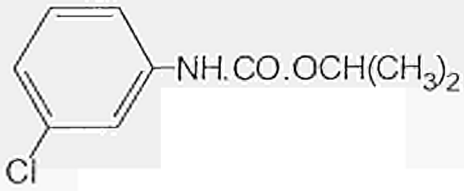
REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF CHLORPROPHAM

(Opinion expressed by the SCP on 26 January 1995)

BACKGROUND AND TERMS OF REFERENCE

Chlorpropham is a residual herbicide and potato sprout suppressant which has approvals for use in all member states. Chlorpropham is the common name for isopropyl 3-chlorocarbanilate (IUPAC). It is often referred to as CIPC or isopropyl N(3-chlorophenyl)carbamate. The structure of the molecule is shown below:



In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the ADI for chlorpropham.

1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

Following oral dosing in rats, chlorpropham was rapidly absorbed and quickly eliminated. Within 24 hours, using ring labelled chlorpropham, approximately 80% of the administered dose appeared in the urine and 3% in the faeces. Using isopropyl labelled chlorpropham, approximately 45% was excreted in urine, 3% in faeces and 20% in [14C]O_2. It was stated that enterohepatic circulation occurred. Chlorpropham levels rapidly declined in tissues of adult rats, with biological half lives generally around 3-6 hours. Highest tissue levels were detected in kidney, intestine, liver, blood and lung. (Ref. 1)

Chlorpropham was metabolised to compounds including sulphate and glucuronide conjugates of chloroaniline derivatives. Although the identity of all metabolites was not confirmed with certainty, the main metabolite appearing in rat urine was considered to be 4-hydroxy chlorpropham. (Refs. 1 and 2)

Experiments were carried out in pregnant rats and chlorpropham was slowly transferred to fetuses from dams. Transfer of chlorpropham via milk to pups was shown to occur. The extent of transfer appeared to be influenced by individual variations in dams. Investigations of intracellular distribution of chlorpropham in livers and kidneys of rats indicated that significant levels were found in the nuclear fraction of the liver, with less in the kidney nuclear fraction. (Refs. 1 and 3)

2 TOXICITY

2.1 ACUTE TOXICITY, IRRITATION AND SENSITISATION

Chlorpropham is of low acute oral and intraperitoneal toxicity. The oral LD_{50} in rats is between 5 and 7.5 g/kg bw, while the intraperitoneal LD_{50} in rats is around 700 mg/kg bw. Clinical signs of toxicity consisted of listlessness, bradypnea, ataxia, haemodacryorrhoea, epistaxis, haemorhinorrhoea and exophthalmos. These signs appeared at 2-4 hours after dosing and reached a peak at 8-48 hours. The main necropsy finding was a marked irritant gastroenteritis. In rabbits the oral LD_{50} is around 5 g/kg bw. In a skin irritancy study in rabbits, only very slight irritancy was observed after 4 hours exposure to 0.5g of chlorpropham. Chlorpropham was not a skin sensitiser in a Magnusson and Kligmann assay. (Refs. 4-8)

2.2 SUBACUTE TOXICITY

In a summary paper on chlorpropham, published in 1959, it is stated that groups of 10 rats were fed chlorpropham for 90 days at concentrations of 20000, 5000, 1250 and 310 ppm in dry casein diet. All the treated groups were reported as surpassing the control group in mean body weight and food consumption. The mean liver weights of the 20000, 5000, and 1250 ppm groups were significantly increased over those of the control group, although no microscopic abnormality was found at termination. (Ref. 9)

Further 90-day studies were performed in rats and mice. The rats were dosed orally at dose levels of 0 (Control), 17, 70, 300 or 1200 mg/kg bw/day. At the two highest dose levels there were significant reductions in red blood cell counts and haemoglobin and haematocrit values and there was also a dose-related increase in the count of morphologically altered red blood cells and the reticulocyte count. In addition, absolute and relative liver and spleen weight were significantly increased at doses of 300 and 1200 mg/kg bw/day. Histological examination showed that chlorpropham increased production of red blood cells in the bone marrow and increased accumulation of red blood cells in the liver and spleen. A NOEL of 70 mg/kg bw/day was determined in rats. In the mouse study, doses of 0 (Control), 105, 210, 420 or 840 mg/kg bw/day were used. As was observed with rats, haematological examinations showed that chlorpropham affected red blood cells, particularly in males. A NOEL of 420 mg/kg bw/day was determined. (Ref. 10)

In a 1960 publication, beagle dogs (2/sex/dose) were fed diets containing 0, 200, 2000 or 20000 ppm chlorpropham for 1 year. An overall slight body weight loss was seen in animals at the top dose level. Food consumption in these animals was correspondingly reduced. There were no deaths and no overt signs of toxicity. Haematology investigations revealed reduced haemoglobin and haematocrit values at 1-6 months in animals at 20000 ppm. Urinalysis revealed no treatment-related findings. At the top dose, liver weights were

moderately increased and spleen weight markedly increased. Histopathological investigations revealed no treatment-related findings apart from splenic congestion in 2 dogs fed at the top dose. The NOAEL was 2000 ppm, equivalent to 50 mg/kg bw/day. (Ref. 11)

2.3 CHRONIC TOXICITY/CARCINOGENICITY

In an experiment of 116 weeks duration, reported in 1972, mice (25/sex/dose) showed no evidence of carcinogenicity when fed diets containing 1000 ppm chlorpropham or when subcutaneously dosed 9 times with 1 g/kg bw chlorpropham. Urethane, the positive control which is structurally related, caused multiple lung tumours (Ref. 12). In a study reported in 1969, 18 mice/sex/strain from 2 hybrid strains were dosed from days 7-28 with 464 mg/kg bw and subsequently administered diets containing 1112 ppm chlorpropham until 18 months. There was no evidence of carcinogenicity. (Ref. 13)

In a 1958 publication, the authors concluded that orally dosed chlorpropham has weak initiating activity in the presence of the promoter croton oil. Propham was also considered to be a weak initiator and urethane had a stronger initiating capability (Ref. 14). In a 1972 publication, there was no evidence of carcinogenic potential in hamsters treated with 2000 ppm chlorpropham or propham. A possible treatment-related increase in testicular atrophy was seen in groups treated with either propham or chlorpropham. (Ref. 12)

In a study reported in 1960, rats (25/sex/dose) were fed diets containing 0, 200, 2000 or 20000 ppm chlorpropham. These animals showed reduced body weight gain but increased food consumption when administered 20000 ppm chlorpropham in the diet. During the last 13 weeks of the 2 year test period, mortalities in male rats were increased at 20000 ppm. Haemoglobin and haematocrit values were reduced mainly at 3 months, and liver and spleen weights appeared to be increased at 20000 ppm. Very few rats were alive at the end of the study. The apparent no effect level was 2000 ppm in the diet, equivalent to approximately 100 mg/kg bw/day. (Ref. 11)

2.4 MUTAGENICITY

In *in vitro* studies chlorpropham was negative in *Salmonella* mutation assays, in a mammalian cell gene mutation assay and in chromosome aberration assays using human lymphocytes. Chlorpropham, however, does have some antimitotic activity in animal and plant cells. Investigative studies indicate that microtubules are not the primary target of chlorpropham action but that the primary site of action may be at the level of microfilaments, with microtubule disassembly resulting from this. (Refs. 15-26)

In *in vivo* studies chlorpropham was negative in a sperm head abnormality assay in mice. (Ref. 27)

Chlorpropham was one of 42 chemicals tested in a range of experiments as part of an evaluation of short term tests for carcinogens in the late 1970's. A report was published of this international collaborative programme in 1981. Conflicting results were seen for chlorpropham and the conclusions of the report state that the responses observed could be interpreted in two separate ways depending on the data selected for consideration. The alleged non-carcinogenicity of chlorpropham was supported by negative responses in 19 different bacterial mutation assays using two different marker organisms and a wide range of

protocols, the negative responses in the two independent UDS assays studied, and three negative responses in separate cytogenetic assays one conducted *in vivo*. Nonetheless, several positive responses were observed in assays sensitive to the carcinogen urethane, which is structurally related to chlorpropham and these activities, especially positive results in two cell-transformation assays, led the group to conclude that chlorpropham should be re-evaluated for carcinogenic properties. Although this suggestion was triggered by the positive transformation responses, it is important to also note that chlorpropham also gave clear positive responses in the yeast aneuploidy and mitotic recombination assays. The report draws attention to the fact that the assays that find chlorpropham positive are, in general, among the least used for screening purposes, those most used finding it negative. A re-evaluation of the carcinogenicity of chlorpropham would present an opportunity either to confirm the need for such assays or to increase reliance on the more traditional ones such as bacterial mutation and cytogenetics. (Ref. 28)

2.5 REPRODUCTIVE TOXICITY

2.5.1 TERATOGENICITY

In a study published in 1990, mated female Wistar rats (25/dose) were administered orally by gavage from day 6 to 15 post coitum with 0, 50, 200 or 800 mg/kg bw/day of chlorpropham (99.3% pure) and killed on day 21. There were no deaths, although overt signs of toxicity were seen at 800 mg/kg bw/day, on nearly every day during the treatment period. Throughout the treatment period, food consumption was significantly decreased at 800 mg/kg bw/day, while at 200 mg/kg bw/day, there was slight decrease from day 6 to 11. Body weight gain was reduced in a dose-related fashion at 200 and 800 mg/kg bw/day mainly during the first half of the dosing period. There were no treatment-related effects on numbers of implantations, pre-and post-implantation losses or numbers of live fetuses. Examination of fetuses revealed no treatment-related affects on sex ratios or any external or visceral malformations/variations related to treatment. At 800 mg/kg bw/day, reduced body weights were observed in both fetal sexes. As a consequence of the delayed development in these fetuses, retarded ossification was noted to a greater extent in some fetuses at 800 mg/kg bw/day. At 800 mg/kg bw/day, a possible treatment-related increased incidence in fetuses with supernumerary ribs was observed. The authors state that this was within the normal range of deviations for the rat strain used. The no effect level for maternal toxicity was 50 mg/kg bw/day, based on reduced body weight gain and food consumption at 200 and 800 mg/kg bw/day. The no effect level for fetotoxicity was 200 mg/kg bw/day based on reduced fetal body weights and associated retarded ossification at 800 mg/kg bw/day. There was no evidence of teratogenicity at any dose level. (Ref. 29)

In a US National Cancer Institute study of 48 compounds over the period 1963-68, chlorpropham was given subcutaneously in DMSO at 1000 mg/kg bw (maximum tolerated dose) to BL6 pregnant mice. Dosing occurred over days 6-14 of gestation, and the animals were killed on day 18. There were only 6 litters. Maternal weight gain, liver weight and the amount of amniotic fluid per fetus were significantly increased. Crown-rump length was decreased compared to controls. The authors did recommend that a further pre-natal study was needed as the study performed was limited by the number of litters in the treatment group. In a post-natal study in BL6 mice incorporating the same dosing regime, neonates were examined at birth and at day 8 and then were killed. There were only 13 litters, and these were compared only with 4 control litters. The average number of live neonates/litter

was low. Mortality between days 1-8 was relatively high. However, control mortalities were also high. As with the pre-natal study, further investigations would be required in order to clearly ascertain the post-natal effects on pups exposed *in utero*. (Ref. 30)

2.5.2 MULTIGENERATION STUDIES

A 2-generation reproduction study was performed on rats at dietary levels of 0 (Control), 1000, 3000 and 10000 ppm. It was concluded that chlorpropham does not affect fertility and reproduction at the doses administered. Spleen and liver weight were clearly affected and reduced weight gain was observed in adults at the highest dietary level, along with increased mortality in F1 offspring. The same dose also produced significantly lower body weights in F1 generation on day 21 and in the F2 generation on days 14 and 21. The high dose level (equivalent to approximately 750 mg/kg bw/day) also caused significantly decreased kidney and spleen weight in F2 males. The NOAEL was 3000 ppm, equivalent to approximately 150 mg/kg bw/day. (Ref. 10)

3 OVERVIEW AND ESTIMATION OF ADI

The only quantitative data relating to no effect levels can be taken from the one year dog and two year rat studies. In each of these studies, dietary levels of 2000 ppm were no effect levels, with relatively non-specific toxicity seen at higher doses. In dogs, a dietary level of 2000 ppm is equivalent to 50 mg/kg bw/day, while in rats the same dietary level equates to about 100 mg/kg bw/day over two years. However, these experiments were conducted some years ago and it is difficult to assess the quality of the studies from the available reports. In view of this inadequacy of the database, and to take account of the resultant uncertainty on the toxicity of chlorpropham, a higher than normal safety factor is considered necessary. Using a 500 fold safety factor, it may be concluded that the temporary Acceptable Daily Intake should be in the order of 0.1 mg/kg bw pending review of a complete up to date database.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF ETHEPHON

(Opinion expressed by the SCP on 26 January 1995)

BACKGROUND AND TERMS OF REFERENCE

Ethephon is the common name for 2-chloroethylphosphonic acid which is a plant growth regulator, which has been marketed in member states since 1971. In the context of its work to establish maximum pesticide residue levels pursuant to the relevant Community legislation, the Commission requested the Scientific Committee for Pesticides to examine toxicological data relating to ethephon and to estimate its Acceptable Daily Intake.

Ethephon is readily absorbed by plants and releases ethylene, which is also a natural plant hormone. Ethylene influences physiological processes such as ripening or maturation. Ethephon was reviewed by the JMPR in 1977, 1978, 1983 and 1985 but no ADI could be estimated because toxicological data were not made available. The Scientific Committee has been requested to establish an ADI, several new studies now being available. Since then the JMPR has reviewed ethephon again in 1993 and an ADI has been estimated. The present evaluation summarises data available to the Scientific Committee which were considered relevant to the derivation of an ADI. The toxicological evaluation of the Scientific Committee has been compared with that of the JMPR.

TOXICOLOGY AND METABOLISM OF ETHEPHON

Physical and chemical properties

The active ingredient is a white solid substance with a purity of 93.4%. This product is a waxy solid which is difficult to handle as such. Therefore a technical product is marketed named Base 250 containing 71.3% of ethephon and 21.7% of water. The main impurities are ethane-1,2-diphosphonic acid (3.5%), mono-2-chloroethylester of ethephon (3%), the mono-2-chloroethylester of 1,2-ethane diphosphonic acid (2.2%), the monoethylether of ethephon (1.5%) and phosphonic acid (0.5%). All dietary concentrations mentioned in this report have been corrected for purity and are expressed as pure ethephon.

The density of the pure compound is 1.6, the melting point 74-75°C and the vapour pressure <2.78 hPa at 25°C. The compound is very soluble in water, methanol, acetone and ethylacetate, but poorly or non-soluble in n-hexane, toluene or dichloromethane. The log Pow(n-octanol/water) is -0.22 at 25°C. The dissociation constants are pK1=2.5 and pK2=7.2. Ethephon is stable at pH=3 and lower. It decomposes, liberating ethylene at

higher pH values and the transformation is almost complete in alkaline medium.

In animal studies ethephon inhibits plasma and erythrocyte cholinesterase, although this is not expected from the chemical structure. It is possible that impurities in the ethephon formulation might cause the inhibition of cholinesterase. Ethephon has never been found to inhibit brain acetyl cholinesterase in any of the animal species tested. Physico-chemical properties could prevent crossing of the blood:brain barrier.

Absorption, distribution and excretion

1.Rat studies

Four groups of five male and five female rats were used. In the first three groups a single dose of 50 mg/kg bw of ^{14}C-ethephon was administered intravenously, orally and orally following 14 oral doses (50 mg/kg bw/day) of non-labelled ethephon respectively. The fourth group received a single oral dose of 1000 mg/kg bw ^{14}C-ethephon. For the low oral dose around 90% of the administered radioactivity was recovered after 120 h, most of it after 16h. In urine and faeces 50-60% and 4-6% were found respectively. In expired air 18-20% was recovered as ethylene. Tissue concentrations were low or non-detectable. When ethephon was given orally at the high dose level, around 86% of the dose was recovered in 120h, most of it being eliminated in 24h. The substance was principally found in urine (47-49%), in expired air (17-20%) and faeces (6.5%). Tissue concentrations were again low or non-detectable at 120h. After pre treatment with non-labelled ethephon the recovery of the labelled substance was comparable with the single dose. Comparison of excretion data after intravenous and oral administration and oral administration indicated that absorption after oral administration was higher than 70%. No major sex differences were observed.

Two additional groups, receiving respectively 50 and 1000 mg/kg bw, were used to determine kinetic parameters. Animals were killed 168h after dosing. Following a dose of 50 mg/kg bw, whole blood parameters indicated that Cmax was rapidly attained (Tmax 0.5-2h). Thereafter, concentrations of radioactivity declined to levels near background in 12-24h. After 1000 mg/kg bw blood levels peaked initially at 0.5h, then again at 4h, thereafter falling rapidly in a similar manner as with the low dose (1).

2.Dog studies

Three dogs were dosed orally with 180 mg/kg bw ^{14}C-ethephon. Urine, blood, expired air (ethylene and CO_2) and faeces were collected at intervals. Ethephon was rapidly absorbed and blood levels decreased from peak levels after 3h to traces after 22h. Ethephon was excreted rapidly in urine and faeces and via air as ethylene. After 72h only traces remained in the tissues. Plasma and erythrocyte cholinesterase activities in blood were depressed, but recovered in a few hours (plasma) or days (erythrocytes) (2).

Biotransformation

Ethylene in expired air has to be considered as a major metabolite in both species tested (rats and dogs). Formation of ethylene is also the transformation route in plants. Following oral

dosing of ^{14}C-ethephon to rats, 4 TLC-peaks were observed in urine. Approximately 50% of the radioactivity was identified as parent compound. Two metabolites were identified as di- and mono-sodium salt and the other remained unidentified. In faeces the same metabolites were found. The metabolism of ethephon was sex, dose and route independent (3).

In the urine of dogs, dosed orally with ^{14}C-ethephon only unchanged ethephon was found.

Acute toxicity studies

The oral LD50 of ethephon (technical or purified) in male mice was 1920 mg/kg bw (5). The oral LD50's in rats were 3730 and 2210 mg/kg bw, equivalent to 2650 and 1570 mg/kg bw pure ethephon for male and female rats respectively (6). The oral LD50 of purified ethephon in female rabbits was higher than 562 mg/kg bw (7). No characteristic clinical signs were observed. Male and female Sprague-Dawley rats were exposed for four hours to aerosol concentrations of 6.1, 3.3 or 2.1 mg/l Base 250. The LC50 for both male and female rats was 4.5 mg/l equivalent to 3.2 mg/l of pure ethephon (8). The acute dermal LD50 of Base 250 in male and female New Zealand rabbits was 1710 and 1390 mg/kg bw respectively (equivalent to 1219 and 991 mg/kg bw of pure substance) (9).

Ethephon was corrosive in a 4-hour skin irritation test in rabbits (10). In a 1-hour test it was irritant showing only erythema (10). Ethephon was tested in 2 sensitisation tests. Sensitisation was not observed in either the Magnusson-Kligman or in the Buehler test (11a and 11b).

Subacute toxicity studies

1. Mouse

In a 28-day study, groups of 10 mice of each sex were fed with ethephon in the diet for 30 days at concentrations of 0, 225, 900, 1800 or 3600 ppm. Ethephon did not induce detectable effects in behaviour, body weight or food consumption, but liver weights were decreased in males at doses of 1800 and 3600 ppm. Red blood cell cholinesterase activity was depressed at 900 ppm and higher in the males and at 450 ppm and higher in the females after 4 weeks. Plasma cholinesterase was depressed also at dosages of 450 ppm and higher. The NOAEL was 225 ppm based on erythrocyte cholinesterase inhibition (12).

In a second 28-day study ethephon was administered to male and female CD-1 mice at concentrations of 0, 30, 100, 300, 1000 and 3000 ppm. No effects were observed in clinical signs or mortality, food consumption, body weight, organ weights or liver enzymatic functions. Plasma and erythrocyte cholinesterase activity was inhibited in both sexes after 2 and 4 weeks of exposure at 1000 and 3000 ppm. Plasma cholinesterase was inhibited in the 300 ppm group at 2 weeks also. Brain cholinesterase was not inhibited at any dose level. The NOAEL, based on erythrocyte cholinesterase inhibition, is 300 ppm, equal to 51 mg/kg bw/day (13).

In a third 28-day study, conducted to establish a maximum tolerated dose, ethephon was administered in the diet of CD-1 mice at concentrations of 0, 3000, 10000, 25000 and 50000

ppm. No effects were observed in the incidence of clinical signs or mortality. Food consumption was reduced in the high dose group at week 1 and body weight reduction occurred in the 25000 and 50000 ppm dose groups throughout the study. Dose-related inhibition of plasma and erythrocyte cholinesterase activity was observed in both sexes at all dose levels after 2 and 4 weeks. No brain cholinesterase inhibition was observed at any dose (14).

2. Rats

In a 28-day study CD-rats were given diets containing ethephon at concentrations of 0, 625, 1250, 2500, 5000 and 10000 ppm. As in mice no incidence of mortality, clinical signs or liver enzymatic dysfunction was observed at any dose in either sex. Plasma and erythrocyte cholinesterase were inhibited dose-relatedly in both sexes. Based on erythrocyte cholinesterase inhibition, the NOAEL for males was 1250 ppm, but an NOAEL could not be established for females (15).

A second rat study was conducted to establish a maximum tolerated dose in CD-rats fed with diets containing ethephon at concentrations of 0, 10000, 25000 and 50000 ppm for 28 days. Decreased food consumption and body weight gain were observed at the two highest dose levels, while animals at the highest dose level had diarrhoea from day 10 onwards. Neither mortality, nor brain cholinesterase inhibition was observed. Inhibition of plasma and erythrocyte cholinesterase was dose related in both sexes (16).

3.Rabbits (dermal)

In the first study male and female New Zealand rabbits received repeated dermal exposures at 300 and 600 mg/kg bw Ethrel (ethephon 39.5% in aqueous solution) per day, 5 days per week for 3 weeks. No systemic toxicity was observed but moderate to severe dermal irritation up to ulceration occurred (17).

In the second study groups of rabbits were exposed to 0, 20, 60 or 180 mg/kg Ethrel (ethephon 31.5%) on intact or abraded skin, 5 days a week for 3 weeks. No clinical or biological changes were recorded except acanthosis in two high dose animals (18).

4.Dogs

Ethephon was administered daily in the diet of male and female beagle dogs at levels of 0, 100, 300, 1000 or 2000 ppm for 52 weeks. Animals at 2000 ppm showed soft stools. There was a significant decrease in terminal body weight and in spleen weight at the highest dose of 2000 ppm. There were no histopathological findings in the spleen and no effect on haematology was reported. The NOEL was 1000 ppm, based on body and spleen weight changes. However, an NOAEL can not be estimated because measurements of cholinesterase activity were not performed (19).

A two-year study with ethephon technical was conducted in beagle dogs at 0, 30, 300 or 1500 ppm in the diet. Initially the highest dose was 3000 ppm, but was decreased to 2000 ppm at week 4, decreased again to 1000 ppm at week 6 and increased to 1500 ppm from

week 25-104. Soft stools and intermittent emesis were the only clinical signs reported in the dose groups of 300 ppm and higher. Plasma cholinesterase activities in treated male and female dogs were decreased in all dose groups, but erythrocyte cholinesterase was inhibited only at 300 and 1500 ppm. There were no effects on brain cholinesterase. Macroscopic post-mortem examination indicated thickened stomach and intestinal wall in dogs from all groups. Histopathologically, smooth muscle hypertrophy in the stomach and small intestines was found at 300 and 1500 ppm. The dose level of 30 ppm, equal to 0.86 mg/kg bw/day is considered as the NOAEL (20).

Special studies on delayed neurotoxicity

Groups of 10 white Vantress hens were intubated with 1000 mg/kg bw/day of either technical or formulated ethephon for 5 days. This dose caused mortality and doses were reduced to 500 and 200 mg/kg bw/day for additional 5 or 9 days. No signs of neurotoxicity were noted and no gross pathology in the spinal cord or sciatic nerve was observed in any of the necropsied hens (21).

Mature White Leghorn hens were dosed with 3850 or 3160 mg/kg pure ethephon. Nearly all animals at 3850 mg/kg died after 24h. In the 3160 mg/kg group signs of lethargy and ataxia were present following dosing. The surviving hens showed no signs of toxicity after 21 days. They were given a second dose of 2370 mg/kg bw and again observed for 21 days. No signs of neurotoxicity or treatment related histomorphological changes in the neural tissues were observed (22).

Long term/carcinogenicity studies

1.Mice

In a study conducted with ethephon 4 groups of Swiss albino mice (85 animals/group/sex) were fed diets containing 0, 30, 300 and 1000 ppm of pure ethephon for 78 weeks. Survival was reduced among males fed 300 and 1000 ppm, but the report claimed that death was due to inter current infections. Plasma and erythrocyte cholinesterase activities were decreased in both male and female animals at 300 and 1000 ppm. Females appeared to be more sensitive than males. Brain cholinesterase activity was not affected. The NOAEL could be established at 30 ppm (equal to 4 mg/kg bw/day). There was no treatment related increase in tumour incidence in this study (23).

A dietary oncogenicity study was conducted exposing male and female CD-1 mice to 0, 100, 1000, 10000 and 50000 ppm ethephon for 78 weeks. Animals dosed at 50000 ppm were sacrificed after 1 week due to excessive morbidity and mortality. These results are in contrast with the 28-day study in mice (14), in which the animals survived 50000 ppm ethephon. At all other dose levels no adverse changes were observed in mortality, clinical signs, haematology and clinical chemistry (except cholinesterase inhibition) or gross and microscopic pathology. Weight gain was depressed at 10000 ppm only. Toxicologically significant cholinesterase inhibition in erythrocytes was observed at 1000 and 10000 ppm while brain cholinesterase activity was unaffected. The NOEL was claimed to be 1000 ppm based on weight gain, but based on erythrocyte cholinesterase inhibition the NOAEL is 100

ppm, equal to 14 mg/kg bw/day. Again, ethephon did not induce treatment related tumours in this study (24).

2.Rats

Ethephon was fed to Sprague-Dawley CD rats at nominal levels 0, 30, 300 and 3000 ppm for two years. No clinical signs or effects on gross or microscopic pathology were observed in any of the animals. Cholinesterase activity was inhibited in plasma and red blood cells at 300 and 3000 ppm. Brain cholinesterase was not affected and there were no signs of CNS effects at any dose level. Ethephon did not increase tumour incidence in the rat. Taking into account the slight cholinesterase inhibition at 300 ppm in females, the NOAEL is 30 ppm, equivalent to 1.5 mg/kg bw/day (25).

Ethephon was administered to Sprague-Dawley CD rats at dietary levels of 0, 300, 3000, 10000 and 30000 ppm for 97 (males) and 104 (females) weeks. Treatment related effects were observed at the two highest dose levels including depression in food consumption, decreased body weight gain and decreased urinary pH. At 30000 ppm kidney weight was increased. Ethephon inhibited plasma and erythrocyte cholinesterase at all dose levels, but at 300 ppm erythrocyte cholinesterase inhibition was less than 20%. No inhibition of brain cholinesterase activity was found. Animals from the two highest groups showed some evidence of recovery when they were maintained for 4 weeks on control diet following 52 weeks of exposure. This recovery was not complete for body weight gain and cholinesterase inhibition. An NOAEL could be established at 300 ppm, equal to 13-16 mg/kg bw/day, based on erythrocyte cholinesterase inhibition. Ethephon was not carcinogenic even at the highest dose tested in this study of 30000 ppm (26).

Reproduction studies

1. Two-generation reproduction

A 2-generation reproduction study has been performed with Sprague-Dawley rats (28 animals per sex per group) receiving dietary concentrations of 0, 300, 3000 or 30000 ppm ethephon. Animals were dosed for 10 weeks prior to breeding for the F1a and F1b generations. Dosing continued throughout mating, gestation, parturition and lactation. The F1b generation was used to produce the F2a and F2b generations under the same regimen. Parental body weights were consistently reduced at 30000 ppm and sporadically affected at 3000 ppm. Similar effects occurred in the pups of both generations. The number of pups dying in the F1b generation was increased at 30000 ppm. Deaths generally occurred in the perinatal period (postnatal days 0-7). The incidence of soft stools was consistently increased at 30000 ppm. Mating, fertility and gestation were not affected by ethephon. The NOAEL is 300 ppm, equivalent to approximately 25 mg/kg bw/day, for reproduction parameters and offspring (27).

2.Rat teratology/embryotoxicity

The teratogenic potential of ethephon was studied in Charles River rats. Pregnant animals (25 per group) were dosed with 0, 200, 600 and 1800 mg/kg bw./day on days 6-19 of

gestation. There were no biologically meaningful differences in appearance or behaviour, mean maternal body weight, caesarean section observations and embryo/fetotoxicity of the rats in the 200 or 600 mg/kg group compared to the control. The dosage level of 1800 mg/kg bw/day produced excessive maternal toxicity, 14/25 animals died during the treatment period and only 9 litters with viable fetuses were available for evaluation. The NOAEL was 600 mg/kg bw/day, based on maternal and fetotoxicity (28).

In another (preliminary) teratology study 12% mortality was observed in females dosed with 600 mg/kg bw/day. The main study was conducted using CD rats (25 per group) dosed by gavage with 0, 125, 250 or 500 mg/kg bw/day on days 6-15 of gestation. No dose related alterations in any maternal or fetal parameter were observed. The NOAELs for maternal and embryo/fetotoxicity were 500 mg/kg bw/day (29).

3.Rabbit teratology/embryotoxicity

New Zealand white rabbits (17 per group) received daily oral gavage doses of 0, 50, 100 or 250 mg/kg bw/day ethephon during days 6-19 of gestation. Animals dosed with 250 mg/kg bw/day showed decreased activity during and after dosing interval. There were 1, 2, 4 and 8 mortalities in the 0, 50, 100 and 250 mg/kg bw/day groups respectively. The mortality in the 100 and 250 mg/kg bw/day groups was considered as treatment related. Animals of the 250 mg/kg bw/day group had a transient decrease in body weight gain but returned to normal during the post-dosing interval. No treatment-related gross lesions were found at necropsy. No differences occurred with regard to the mean number of corpora lutea or implantations. At 100 and 250 mg/kg bw/day an increase in the resorption rate occurred, which was statistically significant at the highest dose only. There were no effects on the fetuses. The NOAEL in this study is 50 mg/kg bw/day for both maternal and embryo/fetotoxicity (30).

In a second study New Zealand white rabbits (22 per group) received daily oral gavage doses of 0, 62.5, 125 or 250 mg/kg bw/day ethephon during days 7-19 of gestation. Treatment-related mortality (17/22) occurred in the 250 mg/kg bw/day group and gross lesions in the stomach were recorded. The principal treatment-related clinical signs in the 250 mg/kg bw/day group before death were ataxia, lethargy, prostration and anogenital staining. Post implantation loss and percentage of early resorptions were considerably higher and number of live fetuses lower at 250 mg/kg bw/day. Transient decreases in body weight gain occurred in the 125 mg/kg bw/day group. No differences were found with regard to the mean number of corpora lutea or implantations, the number of dams with live or resorbed fetuses or in fetal body weight up to 125 mg/kg bw/day. The NOAEL is 125 mg/kg bw/day for both maternal and fetal toxicity (31).

Ethephon did not demonstrate teratogenic potential in any of the studies with rats or rabbits.

Mutagenicity

Ethephon was evaluated in the Ames Salmonella assay. The results were negative with strains TA-98, TA-100, TA-1537 and TA-1538, but positive in strain TA-1535 (base-pair substitution) with and without metabolic activation (32).

All other tests were found negative. No mutagenic activity was observed in three CHO/HGPRT-tests (33, 34 and 35), a chromosomal aberration test in CHO-cells (36), two rat hepatocyte DNA-repair tests (37 and 38), a micronucleus test in mice (39) and a dominant lethal test in rats (40).

According to these results ethephon is considered not to be mutagenic.

HUMAN DATA

Two male individuals received oral doses of ethephon (88%) in propylene glycol ranging from 5.4-220 mg per day over a 7 week period. There were no effects on plasma or erythrocyte cholinesterase throughout the study. Transient feelings of urinary urgency were experienced by both subjects at the beginning of the study (41).

A group of 5 men and 5 women received orally approximately 125 mg technical ethephon per day (divided into 3 doses) by capsule for 28 days. A placebo was given to 3 men and 3 women. Cholinesterase activity was unaffected, but the subjects experienced transient complaints of urgency of bowel movements and urination and an increased urination frequency (42).

A group of 10 men and 10 women received 0.5 mg/kg bw/day of ethephon, divided into 3 doses, orally by capsule for 16 days. They were compared to a group of 10 persons receiving a placebo. Clinical signs and symptoms, haematology and blood chemistry were unaffected by ethephon treatment. There was a statistically significant decrease in plasma cholinesterase activity in the treated group throughout the dosing interval, but the enzyme activity was comparable to control values at the end of the recovery period (day 29). Red blood cell cholinesterase was not inhibited at this dose level (43).

Twenty human volunteers in good health received orally by capsule, without knowing to which group they were assigned, 0 (3 males, 3 females), 0.17 (3 males, 4 females) or 0.33 (4 males, 3 females) mg/kg bw/day of ethephon. The substance was given divided in three daily doses for 15 consecutive days. Clinical signs and symptoms, haematology, blood chemistry and urine volume were not affected by the treatment. Plasma cholinesterase was marginally inhibited throughout the treatment and recovery periods (8 and 15 days and up to day 36) at 0.17 mg/kg bw/day. The inhibition was more prominent at 0.33 mg/kg bw/day. Erythrocyte cholinesterase was unaffected at either dose (44).

OVERVIEW AND ESTIMATION OF ADI

1. Ethephon, a systemic plant growth regulator, is readily absorbed by plants and releases ethylene, which has an influence on maturation of plants. It is also readily absorbed by animals and eliminated rapidly, mainly in the urine as unchanged ethephon (>50%) and in expired air as ethylene (20%). In faeces only a relatively small amount is found. Most of the elimination takes already place within 24 hrs. Apart from ethylene only a small fraction of metabolites (mono- and di- sodium salt of ethephon) are found.

2. Ethephon is an inhibitor of cholinesterase in plasma and erythrocytes, but has never been found to inhibit brain cholinesterase in any of the animal species tested.

3. The acute oral toxicity is low, with an LD50 for rats of >2000 mg/kg bw for pure ethephon. The dermal toxicity for rabbits is moderate, with an LD50 of around 1000 mg/kg bw. This may be caused by the acidic and corrosive properties of ethephon for the skin. The compound is not sensitizing.

4. Ethephon has no teratogenic potential and the no effect level for reproductive effects in a multigeneration study was equivalent to approximately 25 mg/kg bw/day. It is neither carcinogenic in long term studies in rats and mice, nor genotoxic in various mutagenicity studies. No delayed neurotoxicity was found in hens.

5. In subchronic and chronic toxicity studies in mice, rats and dogs ethephon inhibits plasma and erythrocyte cholinesterase. The lowest NOAEL of 30 ppm, equal to 0.86 mg/kg bw/day is found in a two-year dog study. In a chronic mouse study 100 ppm, equal to 14 mg/kg bw/day can be considered as the NOAEL. In the first long term rat study a slight effect on erythrocyte cholinesterase was observed at 300 ppm in females, but in the second (more recent study) no meaningful inhibition was found at 300 ppm, equal to 13-16 mg/kg bw/day, so this level can be considered as the NOAEL for rats. All the NOAELs are based on cholinesterase inhibition in red blood cells.

6. Several studies have been carried out in human volunteers. In the most recent studies a dosage of 0.5 mg/kg bw/day is chosen as the NOAEL, based on the absence of cholinesterase inhibition in red blood cells.

7. The animal studies confirm that the end point examined in the human volunteer studies (cholinesterase inhibition) is the crucial end point for estimation of an ADI for ethephon. It was therefore decided to use the NOAEL in humans of 0.5 mg/kg bw/day as the basis for estimating the ADI, using a safety factor of 10, since the no effect level is derived in humans. Therefore an ADI of 0.05 mg/kg bw can be established. This is the same ADI as was estimated by the JMPR in 1993.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE TOXICITY OF MECARBAM

(Opinion expressed by the SCP on 26 January 1995)

BACKGROUND AND TERMS OF REFERENCE

Mecarbam (S-(N-ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorodithionate) is an organophosphorus insecticide which has approvals for use in some member states. In the context of its work relating to the establishment of community maximum pesticide residue levels in various foodstuffs covered by community legislation, the Commission requested the Scientific Committee for Pesticides to review the ADI for mecarbam, by reference to previous reviews carried out by the World Health Organisation/Food and Agriculture Organisation Joint Meeting on Pesticide Residues.

Mecarbam has been reviewed by the JMPR in 1980, 1983, 1985 and 1986. Data relevant to the establishment of an ADI are summarised below. These summaries have been prepared by reference to JMPR reports, not the original study reports.

1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

Following oral dosing in rats, ethoxy-radiolabelled mecarbam was rapidly absorbed and quickly eliminated. Within 48 hours approximately 92% of the administered radiolabel appeared in the urine, 2% in the faeces and 5% in expired CO_2. There was no accumulation in tissues. (Ref. 1) In further studies in rats, using carbamoyl-labelled mecarbam, approximately 80% of administered radiolabel was eliminated in urine within 48 hours, approximately 3% in the faeces and negligible amounts (0.4%) in expired CO_2. (Ref. 4) The apparent differences in the results of these studies could be associated with the different radio-label position used.

Mecarbam was metabolised in the rat to at least 10 non-conjugated and one conjugated compound. Although the identity of all metabolites was not confirmed with certainty, hydrolysis, oxidative desulphuration and degradation of the carbamoyl moiety appear to be the major metabolic reactions. (Ref. 4)

2 TOXICITY

2.1 ACUTE TOXICITY

Mecarbam has been tested for acute oral and intraperitoneal toxicity. The oral LD_{50} in rats is between 20 and 50 mg/kg bw, while that in mice is around 100 mg/kg bw. The

intraperitoneal LD_{50} in mice is around 120 mg/kg bw. Clinical signs of toxicity were consistent with cholinesterase inhibition. (Ref. 1)

2.2 SUBACUTE TOXICITY

Groups of 15 female rats were fed diets containing 0, 1, 5, 10 or 50 ppm mecarbam for 26 weeks. There were no deaths and weight gain and food intake remained unaffected by treatment. Overt signs of toxicity (muscular tremors) were noted at 50 ppm. Cholinesterase activity was depressed in red blood cells and brain at 10 and 50 ppm. Gross pathology and histopathological investigations revealed no treatment-related findings. The NOAEL was 5 ppm. (Ref. 1)

Groups of 4 male and 4 female beagle dogs were fed diets containing 0, 10, 20 or 50 ppm mecarbam for 26 weeks. There were no deaths and no overt signs of toxicity and weight gain and food intake remained unaffected by treatment. Cholinesterase activity was depressed in red blood cells and brain at 20 and 50 ppm. Gross pathology and histopathological investigations revealed no treatment-related findings. The NOAEL was 10 ppm, equal to 0.35 mg/kg bw/day. (Ref. 1)

Groups of 4 male and 4 female beagle dogs were fed diets containing 0, 1, 5 or 50 ppm mecarbam for 2 years. There were no deaths and no overt signs of toxicity and weight gain and food intake remained unaffected by treatment. Cholinesterase activity was depressed in red blood cells and brain at 50 ppm. Gross pathology and histopathological investigations revealed no treatment-related findings. The NOAEL was 5 ppm, equal to 0.15 mg/kg bw/day. (Ref. 1)

2.3 CHRONIC TOXICITY/CARCINOGENICITY

Groups of 25 male and 25 female Sprague Dawley rats were fed diets containing 0, 1, 5 or 50 ppm mecarbam for 104 weeks. Animals receiving 50 ppm showed reduced body weight gain but food consumption remained unaffected. There was some indication of increased mortality among treated animals, but the small group size precluded adequate evaluation. Cholinesterase activity was depressed in red blood cells and brain at 50 ppm. Gross and microscopic examination of tissues revealed no treatment related findings and there was no indication that mecarbam had any carcinogenic potential. The NOAEL was 5 ppm in the diet, equal to 0.2 mg/kg bw/day. (Ref. 1)

2.4 MUTAGENICITY

In *in vitro* studies mecarbam was negative in a *Salmonella* mutation assay, negative at non-toxic dose levels in a mammalian cell gene mutation assay at the TK locus in L5178Y cells and negative in a DNA repair assay using HeLa cells. In *in vivo* studies mecarbam was negative in a micronucleus test in mice. Results of an *in vivo* metaphase analysis test in rats were inconclusive, but the study design was considered inadequate. (Ref. 2)

2.5 REPRODUCTIVE TOXICITY

2.5.1 TERATOGENICITY

Groups of 24 mated female Sprague Dawley rats were administered orally by gavage from day 6 to 19 post coitum with 0, 1 or 3 mg/kg bw/day of mecarbam and killed on day 20. There were no deaths, although overt signs of toxicity (salivation and body tremors) were seen at 3 mg/kg bw/day and body weight gain was reduced at 3 mg/kg bw/day. There were no treatment-related effects on numbers of implantations, pre-and post-implantation losses or numbers of live fetuses. Examination of fetuses revealed no treatment-related affects on sex ratios or any external, skeletal or visceral malformations/variations related to treatment. Overt malformations were seen in 4 fetuses from a single litter at the high dose, but these were not considered to be treatment related in the absence of effects in any other litters. (Ref. 2)

2.5.2 MULTIGENERATION STUDIES

A 2-generation (2 litter/generation) reproduction study was performed on groups of 25 male and 25 female rats at dietary levels of 0 (Control), 2 and 50 ppm. An investiagtion of teratology was incorporated into this study, in which 5 females per group from the second litter of each generation were killed and examined on day 19 or 20 of gestation. It was concluded that mecarbam does not affect fertility and reproduction at 2 ppm. In contrast, at 50 ppm, there was a decrease in the number of pups born alive, an increased pup loss during lactation and a reduced pup weight at weaning, in both litters of both generations. Reduced weight gain was observed in adults at the high dietary level. Evaluation of fetuses removed by caesarean section did not reveal any teratogenic effects, but did reveal a reduced number of implantation sites and corpora lutea and an increased number of resorbtion sites at 50 ppm. The NOAEL was 2 ppm, equivalent to approximately 0.1 mg/kg bw/day. (Ref. 1)

2.6 SPECIAL STUDIES ON NEUROTOXICITY

The potential of mecarbam to induce delayed neurotoxicity in hens was investigated. These studies revealed no indication of any neurotoxic potential. (Refs. 1 and 2)

3 OVERVIEW AND ESTIMATION OF ADI

At the JMPR meeting in 1980 a temporary ADI was established at 0.001 mg/kg bw. This conclusion was confirmed at the 1983 meeting, but further data were required to be submitted by 1985. These data were not submitted and at the 1985 meeting the temporary ADI was lowered to 0.0005 mg/kg bw. The required data were reviewed at the 1986 meeting and a full ADI was established at 0.002 mg/kg bw.

The available data indicate that the toxicological profile of mecarbam is that of a typical organophosphorus compound. Acute and short term toxicity investigations are considered adequate and mecarbam does not induce delayed neuotoxicity in hens. The long term rat toxicity/carcinogenicity study is small by modern standards and there is no carcinogenicity study in a second species. However, an adequate mutagenicity package is available for mecarbam, which does not raise any cause for concern. In view of this, and taking into account the toxicity of organophosphate compounds in general, the inadequacy of the

carcinogenicity investigations is considered not to be of such great concern. An adequate multigeneration study is available together with an acceptable teratology study in rats. Potential teratogenicity has not been investigated in a second species. Overall it is concluded that the available data do support the establishment of a temporary ADI for mecarbam. However, in view of the inadequacies in the data this should be allocated on a conservative basis. Taking the lowest no effect level of 0.1 mg/kg bw/day, and using a safety factor of 200, would give a temporary ADI of 0.0005 mg/kg bw, pending review of a complete up to date database. This is considered to be a very cautious approach since the lowest no effect level is derived from the multigeneration study in which there is a large difference between the no effect and effect levels.

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OPINION OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE GENETICALLY MODIFIED MAIZE LINES NOTIFIED BY CIBA-GEIGY

(Opinion expressed by the SCP on 9 December 1996)

Background and Terms of reference

In the context of notifications pursuant to Council Directive 90/220/EEC of 23 April 1990[1] on the deliberate release into the environment of genetically modified organisms, the Commission invited the Scientific Committee for Pesticides to express an opinion on the possible adverse effect on the environment of the possible development of insect resistance to Bt - toxin. The Commission requested the Committee to give an opinion on the following two questions:

a) The impact on human health and the environment from the use of the herbicide gluphosinate ammonium on the genetically modified maize lines notified by Ciba-Geigy which will be assessed under Directive 91/414/EEC[2] before authorization is given for the use of the above-mentioned herbicide on maize.

b) Possible development of insect resistance to the Bt-toxin cannot be considered an adverse environmental effect, as existing agricultural means of controlling such resistant species of insects will still be available.

OPINION OF THE COMMITTEE

a) The impact on human health and the environment from the use of the herbicide gluphosinate ammonium on the genetically modified maize lines notified by Ciba-Geigy will be assessed under Directive 91/414/EEC before authorization is given for the use of the above-mentioned herbicide on maize.

1. Glufosinate-ammonium has not so far been authorized for direct application onto maize plants and should not, therefore, be used postemergence on the genetically modified maize without authorization in accordance with the provisions of Directive 91/414/EEC.

2. The use of this herbicide on genetically modified maize plants will raise questions of metabolic pathways, residues safety for consumers and impact on the environment, which should be thoroughly evaluated. The Committee wants to emphasise that the evaluation of metabolites should include studies carried out on genetically modificed maize to fully assess the safety of eventually modified metabolic pathways. The Committee is of the opinion that Directive 91/414/EEC covers all these aspects.

[1] OJ N° L 117, 6.5.1990, p. 15
[2] OJ N° L 230, 19.8.1991, p. 1

3. In the event of an authorization for postemergence use of glufosinate-ammonium on genetically modified maize, the Scientific Committee would like to have the opportunity to comment.

b) Possible development of insect resistance to the Bt-toxin cannot be considered an adverse environmental effect, as existing agricultural means of controlling such resistant species of insects will still be available.

1. On the basis of the currently available data, the Scientific Committee for Pesticides is of the opinion that the possible development of insect resistance to the Bt-toxin[3] arising from the cultivation of maize plants containing the Bt-toxin would not be an adverse effect on the environment; in effect, it would not allow the Bt-resistant cornborer to cause any adverse effect that is not already associated with the non-resistant cornborer.

2. The development of insect resistance to the Bt-toxin would be mainly an agricultural problem. If resistance does develop, Bacillus thuringiensis formulations may not be an alternative measure but other existing pest control and agronomic methods, e.g. control by beneficial insects or by conventional pesticides, are available for use to overcome any potential problems.

3. The speed of possible development of insect resistance to the Bt-toxin in the genetically modified maize will depend on the selection pressure on the pest species affected by factors including :

   - The area grown and the frequency that the transgenic maize is grown
   - The extent of refuge areas for cornborers.
   - The simultaneous cultivation of normal and transgenic maize seeds,
     - ➢ crop rotation,
     - ➢ the maintenance of adequate Bt-toxin levels in the maize throughout the growing period,
     - ➢ further development of genetic transformation of maize plants.

   Resistance management strategies are typically needed during the years of actual use of any pesticide, Bt-sprays included. Therefore, the Committee feels that implications of resistance management, including monitoring for possible development of insect resistance to the Bt-toxin in the genetically modified maize, should be fully considered.

[3] Bacillus thuringiensis endotoxin

FURTHER REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF GENETICALLY MODIFIED MAIZE LINES

(Opinion expressed on 12 May 1997)

BACKGROUND AND TERMS OF REFERENCE

In the context of notifications pursuant to Council Directive 90/220/EEC[1] of 23 April 1990 on the deliberate release into the environment of genetically modified organisms, the Commission invited the Scientific Committee for Pesticides to express an opinion the possible adverse effect on the environment of the possible development of insect resistance to Bt-toxin. The Committee expressed its opinion on 9 December 1996 [i]

Austria submitted to the Commission, in its letter dated 14 February 1997[ii] its intention to prohibit by ordinance the marketing of Ciba-Geigy's maize in Austria from 14 February 1997, pursuant to Article 16 of Directive 90/220/EEC : the Austrian authorities annexed to their letter the reasons for their decision.

The Commission requested the relevant Scientific Committees in March 1997 to give an opinion on the following two questions:

(a) Does the information submitted by Austria constitute new relevant scientific evidence which was not taken into account by the Committee at the time its opinion was delivered ?

(b) Would this information thus cause the Committee to consider that this product constitutes a risk to human health or the environment ?

OPINION OF THE COMMITTEE

The evaluation of the SCP was limited to consideration of the possible risk to the environment arising from the use of genetically modified maize seed. In this context, the Committee identified four items contained in the reasons and the information submitted by the Austrian authorities which it considered of relevance to its remit.

1. Qualitative and quantitative differences between genetically modified (GMO) plants expressing BT-toxin and conventional pesticides.

2. Effects on non-target organisms, particularly in soil.

3. Development of insect resistance.

4. Resistance management.

[1] OJ N° L 117, 6.5.1990, p. 15

1. Qualitative and quantitative differences between genetically modified plants expressing BT toxin and conventional pesticides.

The Committee recognised the complexity of comparing the exposure of a pest to genetically modified plants, where exposure may be prolonged and maintained, and conventionally applied pesticides, with shorter and repeated exposure. The potential for development of resistance could be either accelerated or retarded.

The Committee noted that in the case of genetically modified maize, the effect of the genetically incorporated toxin is more targeted than in the case of a spray application which involve a range of Bt-toxins. The exposure scenario in the case of GMO maize is comparable to multiple applications of insecticides, but the impact of the Bt-genetically incorporated toxin on the environment is considerably lower than traditionally applied pesticides.

2. Effects on non-target organisms particularly in soil

Based on the available information, soil exposure from the GMO maize plants will be less than the exposure resulting from a single conventional spray application including the run-off from plants. Only trace amounts of the toxin can be detected in the roots of the maize and furthermore, normal agricultural practice would involve removal of the greater part of the plant at harvest. The plant remains are often shredded and transformed into silage for use as animal feed at a later stage. Bt toxin concentration can be increased as a result of binding and adsorption by soils[iii], but a recent study [iv] has shown that transgenic plant material did not persist at a high level in the soil. Considering the exposure of the soil ecosystem, and exposure in earthworms or Collembola, the Committee noted that these issues were addressed satisfactorily in the dossier submitted by the notifier [v]This assessment also agrees with the evaluation of the US EPA.[vi]

3 & 4. Development of insect resistance and resistance management.

The Committee stated in its previous report of the 9 December 1996 that resistance management strategies are needed during the years of use of any pesticide, Bt-sprays included. The Committee drew attention once again to the need for effective resistance management, including monitoring on agronomic grounds, to prolong the effectiveness of Bt toxin both in conventional sprays and in genetically modified maize. It also felt that the submission of a satisfactory monitoring and resistance management programme should be a requirement for the authorisation to use genetically modified maize seeds expressing Bt-toxin.

CONCLUSION

The Committee considered that the reasons and information submitted by the Austrian Authorities did not add new relevant evidence to that already considered by the Committee and that none of its conclusions on the risk to the environment were affected by the Austrian arguments.

[i] Report of the Scientific Committee for Pesticides, Opinion expressed on 9 December 1996. In Press.

[ii] Communication for the Austrian Authorities (Ministry of Health and Consumer Protection) of 14 February 1997 to the Commission.

[iii] Tapp, H, & Stotzky, G. 1995: Insecticidal activity of the toxins from Bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and soil clays. Appl. and Env. Microbiol. 61 (5), 1786-1790.

[iv] Palm, C.J., Schaller D.L., Donegan, K.K. and Seidler, R.J., 1996: Persistence in soil of transgenic plants produced Bacillus thuringiensis kurstaki delta endotoxin. Can. J. of Microbiol. 42, 1258-1262.

[v] Ciba-Geigy SA., Ciba Semence, Application for placing on the market a GMPt. Part B1-8, C 1-9 and E 1a - 4 b, Nov., 1994 /Aug., 1996.

[vi] US EPA, 1995. Pesticide Fact Sheet. Bacillus Thuringiensis Cry (B) Delta Endotoxin and Genetic Material Necessary for its Production (Plasmid vector pCIB4431) in Corn. US EPA, Office of Prevention, Pesticides and Toxic Substances. 8.10.95.

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