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Reports of the Scientific Committee for Pesticides

(third series)

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FOREWORD

The Scientific Committee for Pesticides was set up by Commission Decision 78/436/EEC of 21 April 1978 (OJ No 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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(1) Nominated as member 20 October 1986
(2) Elected Vice-Chairman 24 October 1985
(3) Deceased on 27 September 1985
(4) Elected Chairman 24 October 1985
(5) Commission of the European Communities, Directorate-General for Agriculture

REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON CAPTAFOL, CAPTAN AND FOLPET.

(Opinion expressed on 7 July '87)

BACKGROUND AND TERMS OF REFERENCE

In the context of its work to revise Annexes II of Council Directive 76/895/EEC and 86/362/EEC, relating respectively to the fixing of maximum levels for pesticide residues in and on fruit and vegetables and on cereals (1), the Commission invited the Scientific Committee for Pesticides to examine the toxicological data on the phthalimide fungicides captafol, captan and folpet. On the basis of this evaluation, the Commission requested the Committee to estimate, if possible, an acceptable daily intake for each of the compounds and accordingly to consider the appropriateness or otherwise of the existing maximum residue levels.

The levels provided for by Directive 76/895/EEC at the time of writing were:

Maximum levels in mg/kg (ppm)

Captafol:	8:	leaf vegetables
	2:	root vegetables
	5:	other fruit and vegetables
Captan:	15:	fruit and vegetable
Folpet:	15:	cherries, lettuce, raspberries, blueberries, currants, grapes, strawberries
	10:	citrus fruit, pome fruit
	5:	tomatoes
	2:	other fruit and vegetables

DISCUSSION

1. CAPTAFOL

1.1 INTRODUCTION

The fungicide captafol has been evaluated by the Joint FAO/WHO Meetings on Pesticide Residues (JMPR) in 1969, 1973 and 1977 and an acceptable daily intake of 0.1 mg/kg (ADI) was established in 1977 (2). Later it became apparent that all the studies on which the ADI was based had been carried out by Industrial Bio-Test Laboratories. Therefore in 1982 the JMPR (2) replaced the ADI by a temporary ADI of 0.01 mg/kg b.w. and required a number of new studies (semichronic and chronic toxicity, carcinogenicity, reproduction). The ADI was withdrawn in 1985 by the JMPR because it was concluded that captafol is carcinogenic in both rats and mice (2).

1.2 TOXICOLOGIAL ASPECTS

1.2.1 Subchronic toxicity

Two semichronic studies were carried out in the rat by different manufacturers. In the first, many toxic effects were found at the two highest dose levels of 2000 and 8000 mg/kg feed, consisting of clinical signs, reduced food intake and growth, clinico-chemical effects and changes in organ weights. Histopathology revealed treatment related changes in kidneys, liver, skin and stomach. No effects were found at 500 mg/kg diet (ca. 25 mg/kg body weight (b.w.) (3). In the second study, growth and food consumption were decreased at the highest dose level of 3000 mg/kg feed and effects on some organ weights were also found.

Histopathology revealed alterations in the kidneys at 1000 and 3000 mg/kg, consisting of tubular nephrosis in the males. In the females an increased incidence of focal nephropathy was observed. Acanthosis, hyperkeratosis and ulceration were present in the non-glandular region of the stomach at 3000 mg/kg. Because there was a drastic loss of captafol from the diets at the two lower dose levels, it is not possible to establish a no effect level in this experiment (4).

Two chronic studies have been performed in mice. In one study by a manufacturer (5, 6), dose levels of 0, 300, 1000 and 3000 mg/kg feed were used, an increased incidence of lymphosarcomas and total haemangiosarcomas was found at the highest dose level of 3000 mg/kg feed. A number of other effects, especially at the highest dose level, were clinical symptoms of toxicity, marked reduction in survival and effects on the red blood picture. Possibly because of the early mortality no liver cell tumours were found at 3000 mg/kg and the potential development of neoplastic lesions in the duodenum could not be reliably assessed (5, 6).
In the other study carried out by Ito et al. (7) using a different strain of mice and dose levels of 0, 750, 1500 and 3000 mg/kg feed, a high mortality was found especially at the highest dose level of 3000 mg/kg. Growth was inhibited in all dose groups and effects on the red blood picture were present in the two highest dose groups. The main effect was a marked increase in tumour incidence. A dose related induction of haemangio-endotheliomas in the heart and adenocarcinomas in the small intestine was found in all groups. In the mid and high-dose groups an increase in papillomas and squamous cell carcinomas of the forestomach was also observed. Due to early mortality in the high-dose group, the tumour incidence was highest in the mid-dose group for liver carcinomas and haemangiomas of the spleen. Liver carcinomas were also found in the 750 mg/kg group (7).

Unlike the experiment by the manufacturer, no increase in the incidence of lymphosarcomas was observed; the dose levels in the two mouse studies were comparable. The dosages per kg b.w., calculated from the food consumption data were, however, higher in the Ito study.

In a chronic study in the rat, in which dose levels of 0, 75, 300 and 1200 mg/kg feed were used, no effect on survival was observed. Some growth inhibition and effects on biochemical variables in the highest dose group of 1200 mg/kg feed were observed. The main effect was an increase in kidney tumours, especially in the males of the highest dose group. There was also an increase in the occurrence of megalocytic renal cells in males and females in the highest dose group. In the liver of the females of this group the incidence of neoplastic nodules was increased. Females of all groups showed a questionable increase in mammary fibroadenomas. Non-neoplastic lesions like ulcerations, hyperkeratosis and acanthosis were found in the stomach at the highest dose level (8). In a 2-year satellite study with a dose level comparable with the lowest dose group in the chronic rat study no effects were observed (9). This study was carried out because it appeared that especially at the lowest dose levels there was a decrease of the active substance in the feed when it was stored for some time at room temperature. Because this loss was much smaller for the high dose groups and because these groups showed an increased incidence of tumours, the effect is less relevant for the evaluation of the toxicity of captafol.

1.2.2 Mutagenicity

Many mutagenicity studies have been carried out. *In vitro* tests showed that captafol is capable of inducing gene-mutations and chromosomal aberrations. In the presence of metabolic activation (proteins, mammalian blood, cysteine and glutathione) the mutagenic activity is reduced or eliminated in most cases. Captafol is probably detoxified *in vitro* by biological thiols and this reaction is also likely to occur *in vivo*.

In vivo, captafol was negative in a mouse spot-test. An in vivo assay for chromosomal aberrations in bone-marrow in the rat and a micronucleus test in mice were also negative. An increased rate of dominant lethal mutations was observed in two of three studies (10).

1.2.3 Human data

An epidemiological study carried out by one of the manufacturers on 1535 employees involved in the production of captafol showed no significant increase of cancer mortalities (11). However, it is known that captafol has strong sensitizing properties and exposure may lead to sensitization in operators and also in people handling recently treated crops and those on which dried residues remain.

1.2.4 Mechanism of toxic action

Metabolism studies in rats, dogs and monkeys showed that the primary metabolic step is a cleavage into tetrahydrophthalimide (THPI) and tetrachloroethylthiol (TES) moities (12). In recent studies special attention was paid to the metabolism of the TES group. In the urine the major metabolite was 2-chloro-2-methylthioethylene sulphonic acid. To explain the formation of this metabolite the authors assumed that a cyclic sulphonium ion must be formed as a transient intermediate in the degradation of TES. This intermediate is a potential alkylating agent. The authors also suggested that sulphite plays a critical role in the detoxification of TES (12).

1.3 AGRONOMIC USAGE AND RESIDUES

Approximately 3500 metric tonnes active substance of captafol have been used annually in the Community, with the main use being to control Septoria diseases (S. nodorum and S. tritici) on wheat (86%). Alternative fungicides, such as chlorothalonil, prochloraz and propiconazole are available, but their effectiveness needs to be established. This use of captafol generally leads to negligible residues occurring in the grain although they do arise in the straw in the range of 1 to 10 ppm. Evidence has been received that residues of captafol occasionally occur on fruit and vegetables such as pome fruits, grapes, lettuce, endive and potatoes. No residues have been found in total diet studies carried out in two countries (12a).

1.4 CONCLUSIONS

Because captafol caused various types of tumours in both rats and mice it has to be considered as a carcinogen in animals with initiating properties. Although no carcinogenicity was observed in an epidemiological study, potential carcinogenic effects on humans at low exposure levels cannot be excluded and consequently no ADI could be estimated. Therefore, the MRL for captafol should not exceed the limit of determination (at or about 0.05 mg/kg).

In view of the toxicological considerations of captafol mentioned above, the Committee concluded that since the potential hazards to human health associated with the use of captafol as a fungicide cannot be eliminated fully by selective reduction of exposure, the prohibition of the use of captafol should be envisaged.

For these reasons, in general, captafol should not be used. However for cereals, in which residues are known not to occur and where sufficient protective measures can be taken to avoid operator exposure, a temporary derogation could be envisaged until adequate alternatives are available. Likewise, a similar type of temporary derogation can also be envisaged for the use of captafol on a certain limited number of non-edible crops (i.e. certain flower bulbs) for which special protective measures can be taken.

2. CAPTAN

2.1 INTRODUCTION

The toxicity of the fungicide captan has been evaluated by the Joint FAO/WHO Meetings on Pesticide Residues (JMPR) in 1965, 1969, 1973, 1977, 1978, 1982 and 1984 (2). In 1982, it became apparent that most of the studies on which the acceptable daily intake (ADI) was based had been carried out by Industrial Bio-Test Laboratories. Therefore, the 1982 JMPR replaced the ADI (0.1 mg/kg b.w. established in 1973) by a temporary ADI of 0.01 mg/kg b.w. and required additonal studies. These studies were evaluated by the 1984 JMPR and the ADI was re-estimated at the original value of 0.1 mg/kg b.w.

2.2 TOXICOLOGICAL ASPECTS

2.2.1 Chronic toxicity and carcinogenicity

Three carcinogenicity studies have been carried out in both mice and rats. In the 80 week National Cancer Institute (NCI) feeding study in mice with dose levels corresponding to 0, 1143 and 2285 mg/kg b.w., the combined incidence of adenomatous polyp and polypoid carcinoma in the duodenum showed a significant dose related trend. In addition, growth was inhibited at both dose levels (13). In the second study in mice with dose levels corresponding to 0, 857, 1429 and 2285 mg/kg b.w., the incidence of benign and malignant duodenal tumours was increased in all dose groups. A higher mortality was found in the highest dose group, and growth was reduced in a dose related manner in all treatment groups (14). The third study in mice was carried out with lower dose levels, corresponding to 0, 14, 57, 114 and 857 mg/kg b.w. At the highest dose level an increased incidence of non-neoplastic and neoplastic lesions in the small intestine was found, especially in the duodenum. Mice from this group also showed reduced growth. In the case of the 114 mg/kg b.w. treatment no effects were found (15, 16).

In the first rat study (dose levels 0, 25, 100 and 250 mg/kg b.w.), a significant trend for kidney adenomas was found. However, the incidences were not significantly increased. Growth was inhibited at 100 and 250 mg/kg, whereas at 250 mg/kg relative liver and kidney weights were increased, and microscopically a higher incidence of hepatocellular hypertrophy was observed. With 25 mg/kg b.w. no effects were found (17). In the second study, with dose levels corresponding to 0, 126 and 303 mg/kg b.w. captan did not show carcinogenic effects.

Growth retardation was observed in both treatment groups (13). In the third study, with dose levels of 0, 6.25, 25 and 100 mg/kg b.w. no carcinogenic action of captan was observed. At the highest dose level, reduced growth and food consumption and an increased relative weigth was found (18).

2.2.2 Reproduction and teratogenicity

In earlier studies evaluated by the JMPR, teratogenic effects of captan were reported in hamsters, rats and rabbits. No teratogenic effects were seen in mice and monkeys, or in later hamster and rabbit studies (19, 20). In a recent 3-generation reproduction study in rats with dose levels of 0, 25, 100, 250 and 500 mg/kg b.w., growth depression and reduced food consumption were observed at all dose levels. Effects on offspring were found at various dose levels, the most sensitive effect being a reduced litter size and pup weight even at 25 mg/kg b.w. No teratogenic effects were seen (21). Since there was no no-effect level, a new one generation reproduction study was carried out with lower dose levels (0, 6, 12.5 and 25 mg/kg b.w.). A slight reduction in pup and litter weight was observed at 25 mg/kg b.w. In this study 12.5 mg/kg b.w. can be considered as a no-effect level (22).

2.2.3 Mutagenicity

Captan is positive in *in vitro* assays. In the presence of metabolic activation (proteins, mammalian blood, cysteine and glutathione) the mutagenic activity is reduced or eliminated in most cases. Apparently, captan is detoxified *in vitro* probably by biological thiols scavenging the reactive intermediates. Detoxification is also likely to occur *in vivo*. Captan was predominantly negative in *in vivo* assays detecting chromosomal aberrations and gene-mutations: mouse spot-test, heritable translocation test, host-mediated assays, dominant lethal tests and micronucleus tests. In some cases the weakly positive results were not confirmed in repeated experiments (23).

2.2.4 Human data

An epidemiological study was carried out on 134 workers at a captan producing plant. Only two deaths of the 134 workers were caused by tumour conditions (pancreas, thymus); no cancer of the duodenum was found (24).

2.2.5 Metabolism

From earlier metabolism studies in rats, it appeared that captan was split into tetrahydrophthalimide and in trichloromethylthio (TMS) moiety: the latter would appear to be the more important in relation to mutagenicity and carcinogenicity. Captan is also hydrolysed into these two parts in aqueous solution; at pH 8 the reaction rate is much higher than at pH 6 or 7. When thiols, such as S-containing amino acids, are present TMS reacts with the thiol and ultimately thiophosgene is formed. This is further metabolized to CO_2, thiazolidine-2-thione-carbo-xylic acid (TTCA) and thiobis (methanesulphonic acid) and its disulphide monoxide derivative. Three metabolites are found after oral dosing, while after intraperitoneal administration the latter two dithiobis metabolites are not formed. No unchanged captan is found in the urine, while in the faeces a relatively high percentage of unchanged captan is found after administration of a high oral dose level (25).

In a comparative study with rats and mice a number of differences were observed. The elimination rate of 14C-TMS captan in the mouse was greater than in the rat. The rat has a lower gastrointestinal pH and showed a longer gastric retention time. An important difference was found in the percentage of radioactivity found in the duodenum. At the high dose of 250 mg/kg b.w., this was 6.8% and 0.6% for mice and rats respectively.

From a separate study, it was concluded that captan remains stable under the acidic conditions of the stomach, but then degrades or metabolizes readily at the higher pHs of the duodenum. At the low dose of 5 mg/kg b.w., duodenal radioactivity was low for both rats and mice (1.7 and 1.1% resp.) (26).

2.2.6 Mechanism of toxic action

To explain the various findings the following mechanisms of action have been postulated (12). Captan in aqueous solution is readily split into tetrahydrophthalimide (THPI) and the trichloromethylthio moiety ($SCCl_3$) in the form of a reactive compound; this may be the basis for the mutagenic effects found *in vitro*. When S-containing amino acids are present a reaction with this group takes place and the product is further metabolized into "harmless" compounds. This may explain the protective effect of cysteine and glutathione against the mutagenic effects *in vitro*, and probably also the absence of mutagenicity in *in vivo* tests and the absence of carcinogenicity in the rat.
However, there may be only a quantitative difference between mouse and rat. The dose levels in mice at which duodenal tumours were found are much higher than the highest levels tested in the rat, when expressed in mg/kg b.w. From metabolism data, it is known that the percentage of radioactivity in the duodenum is higher for mice than for rats. It also appears that with higher dose levels more of the unchanged captan is passed through the intestines. When a large amount of captan passes from the stomach (low pH) into the duodenum (high pH) the molecule is hydrolized rapidly. In this case, it can be assumed that not enough thiols are present to bind the reactive group, which may react with the DNA of the epithelial cells of the duodenum.

Differences between captan and captafol

For both compounds, the hydrolysis of the molecule into a THPI and a TMS or TES moiety is probably responsible for the *in vitro* mutagenic action. SH-containing amino acids protect against mutagenicity by reacting with the TMS or TES-group. With captafol, however, although the TES-group is bound, metabolites may occur containing a cyclic sulphonium ion which is probably alkylating (12). This might explain why with captafol, kidney tumours are found in the rat and various tumours in the mouse, which are not observed with captan.

2.3 AGRONOMIC USAGE AND RESIDUES

Approximately 2700 metric tonnes active substance of captan have been used annually in the Community, with the main use being on pome fruit (70%) to control scab (*Venturia inaequalis*) and such diseases as *Gloesporium*, *Nectaria* and *Botrytis*. Another important use is on grapes (20%) to control mildew and grey mould *Botrytis cinerea*. The residue data available to the Committee was fully consistent with the usage pattern referred to above, residues occurring frequently on pome fruit, grapes and soft fruit. Captan was not found in the UK total diet studies but it regularly occurs, particularly in the fruit group of the total diet surveys carried out in the USA (12a).

2.4 CONCLUSIONS

The Committee concluded that captan is a carcinogen for the mouse, causing duodenal tumours at the highest dose levels. It is not carcinogenic in the rat. The postulated mechanism of toxic action would indicate that captan has a threshold level for the carcinogenicity in mice of captan. Experimentally, the level of this threshold cannot be determined precisely. Therefore, a larger safety factor for the caclulation of the ADI is proposed.

Based on the no-effect level in the reproduction experiment of 12.5 mg/kg b.w., with a safety factor of 1000, the ADI is estimated at 0.01 mg/kg b.w.

In view of the lower ADI now established, the Committee held the view that the current Community MRL for captan in and on fruit and vegetables should be replaced by MRLs (probably at a lower level) for individual crops. However, in order to enable that to be done, relevant usage and residue data need to be studied in detail.

3. FOLPET

3.1 INTRODUCTION

The toxicity of the fungicide folpet has been evaluated by the Joint FAO/WHO Meetings on Pesticide Residues (JMPR) in 1969, 1973, 1974, 1982 and 1984 (2). In 1982, it became apparent that most of the studies on which the acceptable daily intake (ADI) was based had been carried out by Industrial Bio-Test Laboratories. Therefore, the 1982 JMPR replaced the existing ADI of 0.1 mg/kg b.w. (established in 1973) by a temporary ADI of 0.01 mg/kg b.w. and required additional studies. In 1984, in view of the absence of most of the required data and because of concern over possible teratogenicity in the rabbit, the ADI was withdrawn. In 1986 the required data were available and the JMPR reinstated the temporary ADI of 0.01 mg/kg b.w. (2).

3.2 TOXICOLOGICAL ASPECTS

3.2.1 Subchronic toxicity

Three studies have been carried out in the dog. In a 4-week oral study with dosages from 20 mg/kg b.w., many effects were reported including changes in clinico-chemical parameters and in organ weights at 180 mg/kg and greater. Clinical signs, a reduced food intake and growth were observed in all folpet dose groups (27). Dogs receiving dosages of 790. 1800 and 4000 mg/kg b.w. for 90 days showed many toxic effects including clinical signs, reduced growth and food intake, increased mortality, haematological changes and changes in clinico-chemical parameters and also changes in organ weights often in a dose-related manner in all dosage groups. Histopathological changes were reported in gonads, thyroid, lymphatic and haematopoietic systems and striated muscles (28). Dogs, which were administered oral dosages of 0, 10, 60 and 120 mg/kg b.w. for 1 year, showed a depressed food consumption and growth in the mid and high-dose groups. Clinical chemistry revealed decreased cholesterol, total protein, albumin and globulin values in the mid and high-dose groups. At 10 mg/kg b.w. no effects were found (29).

Two semichronic studies were carried out in the rat. In both studies irritation and other effects in the stomach were predominant. In the first semichronic study in the rat, body weight was decreased and changes in some organ weights were reported at the highest dosage level corresponding to 500 mg/kg b.w. Clinical chemistry revealed decreased protein values, and irritation and histomorphologic alterations in the stomach were seen in the 150 and 500 mg/kg groups. At 50 mg/kg b.w. no effects were found (30, 31). In the other 3-months rat study growth was reduced at dose levels corresponding to 200 mg/kg b.w. and higher.

In addition, increased red cell heamatological parameters (at 400 mg/kg) and a decreased blood protein level (at 200 mg/kg and above) were observed. Another important effect observed was irritation of the mucosal squamous epithelium of the oesophagus and stomach in all dosage groups. In the kidneys of the 200 and 400 mg/kg groups an increase in the number of foci was reported. A no-effect level could not be established (32).

3.2.2 Chronic toxicity and carcinogenicity

Two long term studies in mice and two in rats were carried out. In both mouse studies an increase in the number of duodenal tumours was observed. In the first study mice receiving dosages corresponding to 0, 143, 714 and 1714 mg/kg b.w. revealed an increased incidence of duodenal adenomas and adenocarcinomas in the high-dose and in the mid and high-dose groups respectively. High-dose males also showed an increased incidence of jejunal adenocarcinomas. The incidence of mucosal hyperplasia in the duodenum was increased in all folpet dosage groups. Mucosal hyperplasia of the jejunum and ileum occurred more frequently in the high-dose group. A variety of other effects including affected growth, haematological effects, squamous papillomas in the stomach, nodular hyperplasia in the liver, effects on the haematopoietic system and cutaneous proliferative changes were reported. A no-effect level could not be established (2, 33, 34).

In a second study mice received dosages corresponding to 0, 143, 714 and 1430 mg/kg b.w. for the first 21 weeks and 0, 142, 500 and 1000 mg/kg b.w. for the remaining 83 weeks. The incidence of carcinoma of the duodenum and of papilloma and squamous cell carcinoma of the stomach was increased in all folpet treated groups. The increased incidence of tumours of the stomach was possibly due to mechanical obstruction caused by the duodenum tumours. Other effects consisted of reduced life expectancy and growth, clinical signs of toxicity associated with the integumentary system and several affected relative organ weights. Macroscopy revealed lesions of the skin, duodenum, jejunum and stomach. Histopathology revealed also non-neoplastic changes including hyperkeratosis of the skin, oesophagus and non-glandular mucosa of the stomach and mucosal hyperplasia in the duodenum and jejunum. A no-effect level could not be established (35, 36).

In the rat studies effects on the stomach were also observed. In the first rat study (dose levels corresponding to 0, 10, 40 and 160 mg/kg b.w.) no carcinogenic action of folpet was observed. The high dose group demonstrated a reduced growth and an increased incidence of stomach lesions. With 40 mg/kg b.w. no effects were found (37, 38). In the second study with dose levels corresponding to 0, 25, 50 and 100 mg/kg b.w. a reduced growth was seen in all treatment groups. Amongst organ weights, heart, brain and testes were increased at 100 mg/kg and testes weight was also increased at 50 mg/kg. Histopathology revealed lesions of the oesophagus and stomach and foci or areas of cellular alterations in the liver were reported in the mid and high-dose groups. A dose of 25 mg/kg b.w. was a marginal no-effect level (39).

3.2.3 Reproduction and teratogenicity

In earlier studies evaluated by the JMPR (2), teratogenic effects of folpet were reported in chicken eggs and hamsters whilst no such effects were reported in rabbits.

In a recent 2-generation reproduction study in rats with dose levels corresponding to 0, 10, 40 and 180 mg/kg b.w., growth depression and reduced food consumption was reported in the high-dose adults of the F0 and F1b generation and in all high-dose litters. No effects were noticed at 40 mg/kg b.w. (40).
No teratogenic effects were noted in an oral study in rats receiving dosages varying from 10 to 360 mg/kg b.w. Clinical signs of toxicity and reduced weight gain occurred in dams receiving 360 mg/kg (41, 42). In a second study in rats receiving 0, 150, 550 and 2000 mg/kg b.w. clinical symptoms and maternal mortality occurred in the high-dose group. A reduction in maternal weight gain, foetal weights and foetal crown-rump length was reported at 550 and 2000 mg/kg. Skeletal examination revealed significant evidence of delayed ossification of the cranial bones, sternabrae and pubes. Angulated ribs were observed in all folpet treated groups. The foetal no-effect level is below 150 mg/kg b.w. (43, 44).

Rabbits intubated with folpet increased maternal mortality at the highest dose of 60 mg/kg b.w. Maternal body weight gain and food consumption were reduced at 20 mg/kg and above as were mean foetal body weights. A significant increase in the incidence of a so-called "hydrocephaly" (or severe dilatation of the lateral ventricles) was reported at 60 mg/kg. No effects were noted at 10 mg/kg b.w. (2, 45). In an additional study rabbits were given an oral dose of 60 mg/kg b.w./day during end of four consecutive three day periods during gestation, in order to demonstrate whether the association of "hydrocephalus" and maternal toxicity was specific to a unique developmental period.

Maternal toxicity, expressed as death, abortion, decreased food consumption and body weights were reported. Internal "hydrocephalus" occurred in two foetuses. No connection was made between the presence of "hydrocephalus" and the experimental design and no arguments were advanced which could support the authors conclusion that the occurrence of this malformation was not induced by the substance. Therefore, it must be concluded that folpet showed teratogenic effects in this study (46).
Pregnant rabbits receiving 40 and 160 mg/kg b.w. showed a reduced body weight gain. At 160 mg/kg post-implantation loss was increased and gravid uterine weight and mean foetal weight were reduced. Additionally, the number of "small" foetuses was increased at 160 mg/kg. Skeletal examination revealed developmental retardation (reduced ossification) and an increased number of foetuses with a 13th (lumbar) pair of ribs or 13 thoracic vertebrae and 13 thoracic ribs at 160 and 40 mg/kg. No effects were observed at 10 mg/kg (47, 48).

From the teratogenicity studies it is concluded that folpet has potential teratogenic properties in rabbits at a dose level of 20 mg/kg b.w.

3.2.4 Mutagenitiey

Folpet is positive in in vitro assays using bacteria, yeast and cultured mammalian cells detecting gene mutations, chromosome aberrations and DNA damage. In the presence of metabolic activation, blood, cysteine or glutathione, the mutagenic activity is often reduced or eliminated. Apparently, folpet is detoxified in vitro probably by biological thiols scavenging the reactive intermediates.

Folpet was negative in several *in vivo* assays detecting chromosomal aberrations or gene-mutations: a mouse spot-test and tests for micronuclei and chromosome aberrations in bone marrow. Gene-mutation tests with *Drosophila melanogaster* were predominantly negative. Out of five dominant lethal tests, two (by the same author) were positive and three were negative (49).

3.2.5 Mechanism of toxic action

^{14}C-carbonyl labelled folpet is rapidly absorbed, metabolised and excreted in rats. Approximately 95% of the ^{14}C-activity is excreted in the urine. Folpet is easily split into phthalimide and a trichloromethylthio moiety. Phthalimide is mainly metabolised to phthalimic acid and phthalic acid (50). In earlier studies a wide range of degradation products of the trichloromethyl moiety, such as carbonyl sulfide, thiophosgene and sulphur in several oxidation rates, were reported (2).

Based on the chemical structure and the similarity of the toxicity data, the same mechanism of action is proposed for folpet as for captan. Both compounds contain the trichloromethylthio moiety ($SCCl_3$) which is formed after hydrolysis and is bound to glutathione. Glutathione also showed a clear protective effect against mutagenicity of folpet *in vitro*. *In vivo* this mechanism is also likely to occur. This may explain the low toxicity of folpet.
The substance is not carcinogenic in the rat, but induces tumours in the duodenum, jejunum and stomach of the mouse. Dose levels expressed on a mg/kg b.w. basis were much higher in mice than in rats. Moreover, it can be assumed that as for captan, the percentage of the unchanged active substance present in the duodenum is higher in mice at the same dose level than in rats.

3.3 AGRONOMIC USAGE AND RESIDUES

Approximately 4,400 metric tonnes active substance of folpet have been used annually in the Community, with the main use being on grapes (90%) to control downy mildew (*Plasmopara viticola*) and secondarily to control *Botrytis cinerea*).

Folpet residues have not been observed in any of the United Kingdom total diet studies. Occasionally, residues of folpet have been found in strawberries, grapes and pears but in no case did the levels exceed one-half of the relevant maximum residue level (MRL). In the Netherlands, a few samples of blackberries and gooseberries contained folpet just above the MRL (12a).

3.4 CONCLUSIONS

Folpet is carcinogenic for the mouse, causing duodenal tumours at the highest dose levels. It is not carcinogenic in the rat. The postulated mechanism of toxic action would indicate that there is a threshold level for the carcinogenicity of folpet in mice but this threshold level cannot be determined precisely experimentally. Because of these uncertainties a larger safety factor for the calculation of the ADI is proposed. Based on the no-effect level in the dog studies of 10 mg/kg b.w., with a safety factor of 1000, the ADI is estimated at 0.01 mg/kg b.w.

In view of the ADI now established, the Committee held the view that the present Community MRLs for folpet on fruit and vegetables are not acceptable under current conditions and should be reviewed. In order to enable that to be done, relevant usage and residue data need to be studied in detail.

GENERAL CONCLUSIONS FOR CAPTAN AND FOLPET

The phthalimide group of fungicides should be regarded as two sub-groups, captafol on the one hand and captan and folpet on the other.

For captan and folpet, a carcinogenic effect is found in mice but in this case the proposed mechanism of action (see pages 13 and 14) indicates a threshold level for the effect. Therefore, ADIs could be estimated for both captan and folpet using a higher than usual safety factor. For each compound the ADI is 0.01 mg/kg b.w. However, because of the similarity of mechanism of their toxic action, it is proposed that the total intake of captan and folpet together should not exceed 0.01 mg/kg b.w.

It will be necessary to examine closely the current usage patterns and residue level of captan and folpet and their relevant maximum residue levels to ensure that intakes remain below the ADI.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF LINDANE AS AN INSECTICIDE AND ITS RESIDUES IN FOODSTUFFS

(Opinion expressed on 17 February 1988)

BACKGROUND AND TERMS OF REFERENCE

The use of lindane is authorized in certain Member States as an insecticide for use on a wide range of fruit and vegetable crops and for the control of ectoparasites on some animals. Most of the available toxicological data are old and based on tests not carried out to modern protocols. This is particularly the case for the data used by the Joint FAO/WHO Meeting on Pesticide residues (JMPR) in 1977 (1) to establish the current acceptable daily intake (ADI) (0.01 mg/kg b.w.). However, since then the results of new toxicological studies have become available.

The use pattern for lindane has changed in recent years and its usage is now low, having been largely superseded by newer insecticides. Nevertheless, the Commission considers it prudent in the circumstances to request the Scientific Committee for Pesticides to examine the toxicology of lindane and its environmental impact and, on the basis of the former, to estimate, if possible, an acceptable daily intake and accordingly to consider the appropriateness or otherwise of the existing Community maximum residue levels.

The maximum levels provided for by Directives 76/895/EEC, 86/362/EEC, 86/363/EEC (2) at the time of writing were:

Maximum levels in mg/kg (ppm)

2.0: leaf vegetables
0.1: carrots
1.5: other fruit & vegetables
0.1: cereals (applicable 1st January 1990)
2.0: sheepmeat*) applicable
1.0: other meat products*) 30 June 1988
0.008: milk**)

DISCUSSION

1. TOXICOLOGICAL ASPECTS

1.1 Acute toxicity

Lindane shows a moderate acute oral toxicity with a LD_{50} for rats of 88 - 300 mg/kg body weight with the main effect being on the central nervous system (3, 4, 5, 6)

1.2 Irritation

Lindane is an irritating but a non-sensitizing agent. The irritation potency depends on the type of formulation and the mode of application. However, no major problems need occur when good application practices are followed and formulations containing fine dust are avoided, particularly in closed spaces (3).

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* in mg/kg (ppm) of fat contained in meat.

** in mg/kg (ppm) for raw and whole cows' milk assuming a fat content of 4%. For other milk and milk products covered by the Directive the following apply:

- products with a fat content of less than 2% by weight, the maximum level is taken as half that set for raw milk and whole cream milk;
- products with a fat content of 2% or more by weight, the maximum level is expressed in mg/kg of fat. In such cases, the maximum level is 25 times that set for raw milk and whole cream milk.

1.3 Short term toxicity

Two recent semichronic toxicity studies in rats are available, the results of which are not in full agreement (7, 8). In both studies effects on livers and kidneys were observed. Based on the most sensitive parameter, the liver enzyme inductive potency, 2 mg/kg feed was considered as a no observed effect level. In a semichronic inhalation study in rats, 0.1 mg/m^3 was established as a no observed effect level(9).

1.4 Carcinogenicity and mutagenicity studies

Several limited chronic toxicity studies were available (10, 11, 12) the results of which were in agreement with the profile of toxicity arising from the semichronic studies mentioned above (7, 8, 9). In the carcinogenicity studies in mice, liver tumours were found only at high dose levels (300 mg/kg feed) (10, 11). This effect was not apparent in the available studies in rats (10, 11). Additional short-term studies in mice did not indicate genotoxicity (14, 15). Further support for the absence of genotoxicity is provided by the data on mutagenicity: lindane was examined in a variety of test-systems using different endpoints, plants, bacteria, yeast, Drosophila, mammalian and human cells in vitro, as well as whole mammals. It did not induce mutations in any of the systems examined (17, 18, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30), whereas some cytogenetic damage was observed in mammalian and human cells in vitro (11, 16, 22).

The compound is a mitotic poison in plant-systems, thereby inducing C-mitosis, polyploidy and chromosome aberrations (11). The increase in liver tumours observed in the mouse studies is not considered sufficient evidence for carcinogenicity of lindane. The increase of adenomas and carcinomas in the mouse liver is probably associated with the enzyme induction effects of lindane in the liver; this may be due to a promotor effect.

1.5 Acceptable daily intake - Human exposure to Lindane

Since the existing acceptable daily intake (ADI) of 0.01mg/kg b.w. (1) is not based on adequate studies, the Committee considered that a reevaluation of the ADI is necessary. In general, estimation of an ADI requires an adequate chronic toxicity study in rats. Although the Committee was aware that such a study is in progress, it was of the opinion that in the case of lindane available experimental data on the chronic toxicity and carcinogenicity (10, 11, 12, 14, 15)in combination with the results of the two more recent adequate semi-chronic toxicity studies (7, 8) are considered to provide a sufficient basis for establishment of an ADI using a safety factor of 100. From the semi-chronic toxicity studies in rats (7, 8) a no-effect level of 2 mg/kg feed (0.1 mg/kg b.w.) can be concluded . This results in an ADI of 0.001 mg/kg b.w. (60 ug/day/person).

The main source of human exposure is from the diet, particularly from foodstuffs of animal origin. This includes residues from direct agricultural use and also extraneous residues in the food from environmental sources. In the Netherlands, the daily dietary intake during the period 1976-1978 ranged up to 16 ug/person with a mean value of 2 ug/person (31, 32). Studies in the United Kingdom showed levels of lindane of 4, 2.5 and 1.5 ug/kg in the diet in 1966-67, 1975-77 and 1981 respectively (33), corresponding to 4.5 - 12 ug/person. Both data sources indicate low dietary exposure to lindane when compared to the acceptable daily intake of 60 ug/person .

In addition, the possibility exists of localized intake mainly by inhalation from other sources, such as wood preservative treatments and household insecticide use. For instance, information is available that levels of up to 60 ug/m^3 can be reached in poorly ventilated houses where lindane has been used for remedial wood treatment (34, 35). This level is very close to the no effect level of 100 ug/m^3 found in a semi-chronic inhalation study with rats (9). Similar data have been obtained after carpet treatments (36).

Since less volatile alternative compounds are available, the use of lindane for remedial wood preservation and household insecticidal purposes should be discouraged.

2. AGRONOMIC USAGE AND RESIDUES

Approximately 2 500 metric tonnes active ingredient of lindane are used annually in the Community mainly as soil, seed and wood treatments.

Residues of lindane occur, usually at low levels, in many foodstuffs, especially vegetables, cereals and foods of animal origin. The maximum residue levels provided for by Directives 76/895/EEC, 86/362/EEC and 86/363/EEC (2) were based on data arising from uses prevalent many years ago. In the light of the reduced ADI and especially the changed usage patterns, the Committee concluded that the current MRLs should be replaced (probably at lower levels) by MRLs for individual crops.

However, in order to enable this to be done, relevant current usage and residue data would need to be studied in detail. Meanwhile, the current MRLs should continue in use.

3. ECOLOGICAL ASPECTS

3.1 Environmental contamination

Lindane is known to be quite toxic to aquatic organisms with, for example, an LC_{50} - 96 hours - of 2 ug/l and 152 ug/l for Salmo trutta and Carrassius auratus respectively (37, 38, 39, 40). In addition, fish food organisms, such as small crustaceans are very susceptible (38). Therefore, the main concern is to avoid application, handling or disposal practices, such as aerial application or improper disposal which could give rise to spray drift or runoff into water. In this respect, it is reassuring that the current uses in the Community are mainly as superficial soil treatments against soil borne insects and seed treatments against similar insects: there are also localized treatment uses, for instance, in forestry and on felled timber. Furthermore, the use of lindane on aerial parts of plants has been replaced to a large extent by newer insecticides.

Lindane can be found in soil, air and water and on plants, far from areas of contamination/application due to its mobilisation from soil and escape into the atmosphere. However, contamination of the environment seems to be more related to improper disposal of waste arising from the manufacture of lindane which contains the other isomers, especially the alpha and beta isomers. In addition, disposal of the waste arising from sheep dipping and wood treatment may cause localised contamination of the environment by lindane.

Lindane is much less persistent in soil when compared to most other organochlorine pesticides, having a dissipation time (DT_{50}) in the range of 100 - 700 days (41). Dissipation time is highly dependent on such factors as volatilization, soil treatment techniques, soil types, moisture content, temperature and soil crop coverage. However, under anaerobic conditions (eg flooded fields) much higher dissipation rates have been reported (42, 43, 44). Leaching of lindane in soil does not pose a problem and investigations have shown that measurable amounts of lindane will very rarely be found below 50 cm following application to top-soil layer 0 - 10 cm (45, 46, 47, 48, 49).
Significant contamination of surface water will not occur following judicious use of lindane. However, in this context, it is necessary to explain reports of contamination levels of between 20 and 130 ng/l gamma HCH in the rivers Rhine and Meuse (in the Netherlands) for the period 1974-82. Since the Rhine samples also revealed contamination with other isomers of HCH, it is reasonable to surmise that the pollution was of industrial rather than of agricultural origin (50). Rain-water has also been reported to contain residues of gamma HCH. For instance in 1986, the total annual deposit of gamma HCH in The Netherlands was reported to be in the range 200-250 ug/m^2 (51, 52, 53); the source of this contamination remains unknown.

After consideration of the relevant environmental data on lindane, the Committee concluded that, within the Community, the potential environmental hazards seem to be more related to improper disposal of HCH waste than to the current use of lindane in agriculture and in some other fields.

3.2 Bio-accumulation - Food chain effects

In contrast with several other organochlorine compounds, such as DDT, dieldrin, heptachlor and HCB, lindane can only be considered as moderately bio-accumulative and furthermore no distinct food-chain effects on either terrestrial or aquatic organisms have been reported to arise from current agricultural practice, due to the lower rate of bio-accumulation and the relatively quick elimination of the compound. In products of animal origin, lindane is transferred to a lesser extent than any other organochlorine pesticide, with the exception of methoxychlor. The transfer coefficient for lindane in cows' milk, expressed as a percentage of the daily intake excreted in the milk, ranged in various studies between 1.5 and 2.9%. The corresponding coefficients for dieldrin and heptachlor epoxide were reported in the range 17.1-25.8% and 21.9-32.6% respectively (54, 55, 56). Chickens fed for 52 days with 0.3 mg/kg lindane in the feed reached a plateau of 1 mg/kg in the fat, equivalent to a ratio of 1:3. Corresponding ratios for DDT and heptachlor epoxide were reported at 1:10 and 1:13 respectively (57).

4. CONCLUSIONS

The Committee noted the incidence of liver tumours in mice but found it reassuring that this effect was only evident in mice at high dose levels and that no genotoxic or mutagenic effects have been reported. Based on the no-effect level in the rat studies of 0.1 mg/kg b.w., with a safety factor of 100, the ADI is estimated at 0.001 mg/kg b.w. In view of the ADI established, and more particularly the changed usage pattern, the Committee concluded that the current Community MRLs should be reviewed. In order to enable this to be done, relevant up-to-date usage and residue data need to be studied in detail.

With respect to the environmental impact of lindane, the Committee concluded that there is a potential danger of toxic effects to aquatic ecosystems but that this could be sufficiently controlled by adherence to practices which avoid spray drift and runoff into water. The Committee was satisfied that lindane, as currently used in agriculture and some other fields, shows no tendency to leach into groundwater or to accumulate in terrestrial or aquatic food-chains.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THIRAM

(Opinion expressed on 25 May 1988)

BACKGROUND AND TERMS OF REFERENCE

In the context of its work to review Annex II of Council Directive 76/895/EEC relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables (1), the Commission invited the Scientific Committee for Pesticides to examine the toxicological data on thiram. On the basis of this evaluation, the Commission requested the Committee to estimate, if possible, an acceptable daily intake for the compound and accordingly to consider the appropriateness or otherwise of the existing Community maximum residue levels.

The levels provided for by Directive 76/895/EEC at the time of writing were:

Maximum levels in mg/kg (ppm)

3.8: strawberries and grapes
3.0: other fruit and vegetables.

DISCUSSION

1. INTRODUCTION

In 1985 the existing temporary acceptable daily intake (ADI) of 0.005 mg/kg body weight was withdrawn by the Joint FAO/WHO meeting on Pesticide Residues (JMPR) because of the lack of adequate toxicological data. The reevaluation in 1987 showed that the newly submitted data raised additional questions, particularly concerning mutagenicity, teratogenicity, reproduction and neurotixicity. The JMPR was unable, on the available data, to estimate an ADI in 1987 (2).

2. SUMMARY OF TOXICOLOGICAL DATA

2.1 Metabolism

Thiram is absorbed rapidly in the gastro-intestinal tract and excreted mainly unchanged in the urine and faeces. Formation of dimethyldithiocarbamic acid, CS_2, H_2S and dimethylamine have also been described.

2.2 General toxicity

Thiram has a low acute oral toxicity, with an LD_{50} for rats of 1800 - 4000 mg/kg body weight (2, 3). Animal studies have shown it to be irritating to the eye (5) but not to the skin (6), whilst moderate sensitivity has been shown in guinea pigs (7). For humans, the substance must be considered as both irritating and sensitising.

In a semi-chronic study in rats many toxic effects were found at the dose levels of 1.000 and 2.500 mg/kg diet with increased mortality, decreased body weight and food consumption, decrease in lymphocytes and increase in neutrophils, increase in both urea and transminase activity in blood. The relative weights of spleen, testes and thyroid were increased. Histopathology revealed both increased haemosiderosis in the spleen and tubular degeneration of the testes with the presence of atypical spermatids in the epididymus. Even at the lowest dose level of 500 mg/kg diet the effects on body weight, food consumption and relative spleen, testes and thyroid weights were still evident (3,4).

In a second semi-chronic study in rats, decreased body weight and increased activity of transaminases were found at 600 mg/kg feed. At this dose level a slight cholangiolitis was observed. In this study no effect was found at 300 mg/kg diet (8).

In a chronic 18-month feeding study in rats, body weight gain and food intake were decreased at all dose levels (corresponding to 5/6, 20/25 and 50/65 mg/kg body weight per day for males and females respectively). One animal at the mid-dose and four at the high dose developed hind-limb ataxia. The clinical signs were comparable to those described in the semi-chronic studies with the addition of fatty infiltration of the pancreas occurring in a dose-related manner. In the rats showing ataxia, histology revealed demyelinisation and degeneration of the axis cylinders of sciatic nerves (3, 4).

2.3 Reproduction

Studies on reproduction are inadequate due to the absence of a two generation reproduction study. However, results from preliminary studies suggest that thiram may have an effect on reproduction in males and females at high dose levels (4, 10).

2.4 Teratogenicity and embryotoxicity

Teratogenicity studies gave contradictory results. Oral administration to mice at 10 to 30 mg/kg body weight per day from the 6th to the 15th day of gestation resulted in teratogenic and embryotoxic effects (cleft palate, micrognathy, kyphoses, wavy and distorted ribs and wavy and blockshaped bones of the extremities) at all dose levels with a linear dose-effect relationship. Furthermore, 30 mg/kg body weight on day 12 and 13 of gestation resulted in an increased overall level of malformation compared with the same dose level administered from day 6 to 15 (9). In another study on mice, dose levels of 100 and 300 mg/kg body weight administered from day 6 to 14 of gestation showed only slight teratogenic and embryotoxic effects. However, the experiment is difficult to interpret in the absence of a clear dose response relationship; in addition, 20% maternal mortality occurred at the high dose.

In a teratogenicity study on rats, maternal toxicity was observed at all dose levels (40, 90, 136, 164 mg/kg body weight administered from day 6 to 15 of gestation). At the two highest dose levels increased resoprtions were observed, and foetal body weight was decreased at all dose levels. The increase in total anomalies that occurred may be regarded as embryotoxic effects at maternal toxic dose levels. Definite teratogenic effects were not observed (3, 10). In a second teratogenicity study in rats using dose levels of 0, 12.5, 25, 50 and 100 mg/kg body weight on day 6 to 15 of gestation, maternal and foetal toxicity was observed at dose levels of 25 mg/kg body weight and higher. Several embryotoxic effects were found at these dose levels. Anomalies affecting the skull were rather high in all experimental groups (including the saline control group but not in the methyl cellulose control group) without any dose response. The possible effect on skull retardation at 12.5 mg/kg body weight is doubtful in view of discordant results between the two negative control groups (11). It seems questionable whether 12.5 mg/kg body weight can be regarded as the embryotoxic no effect level.

Recently a third teratogenicity study in rats has been received, using dose levels of 0, 7.5, 15 or 30 mg/kg b.w. on day 6 to 15 of gestation. Maternal and foetal toxicity was observed at 15 and 30 mg/kg (alopecia, decreased body weight in the dams; lower foetal weight and retarded foetal development). At 7.5 mg/kg a slight growth inhibition of the dams and a lower placental weight were the only effects. This level can be considered as a dose without effect (26). Also a recent teratogenicity study in rabbits has become available, using dose levels of 0, 1, 2.5 or 5 mg/kg b.w. on day 6 to 19 of gestation. A slight decrease in maternal body weight gain was observed at 5 mg/kg (27). This level can be considered as a dose without embryotoxic or teratogenic effect.

2.5 Mutagenicity and carcinogenicity

Many tests have been carried out to investigate the mutagenic potential of thiram with positive and negative finding being reported in several *in vivo* and *in vitro* test systems.

Thiram was mutagenic in a number of tests with bacteria (12, 13, 14, 28) and Aspergillus nidulans (12, 15). It was positive in one gene mutation assay in mammalian cells (16), but negative in the other (17). Tests for chromosomal aberrations gave positive results in one at cytotoxic dose levels (18) and negative results in another test (23). Metabolic activation in in vitro tests generally seemed to weaken the mutagenic effect. An anaphase-telophase test in CHO-cells and an unscheduled DNA synthesis (UDS) test in rat hepatocytes were negative (19, 20).

In vivo tests also gave contradictory results. Thiram was positive in two micronucleus tests (16, 19), a sperm abnormality test (12) and a test for chromosomal aberrations in mice spermatocytes (29), but negative in another micronucleus test (24). In many cases the purity of the thiram tested is not known (13, 15, 19, 29) or the test was carried out with a formulation (14, 16).

A two-year carcinogenicity study in rats using dose level of 500 and 1000 mg/kg diet did not show enhanced tumour incidence (21). In a further two-year study with rats of the same strain, 500 mg/kg diet again showed a similar result. However, in this study, the addition of 2.000 mg/kg nitrite clearly induced tumours of the nasal cavity and papillomas of the forestomach by the formation of a N-nitroso derivative (22).

3. ENVIRONMENTAL ASPECTS

Thiram may be degraded in water by light and dimethylnitrosamine (DMNA) can be formed when nitrites are present. Furthermore, DMNA is formed at significant levels in Daphnia and fish (Cyprinus carpio) when thiram and nitrites are added to the aqueous medium. In a short-term laboratory test, increased toxicity to fish (Brachydanio rerio) was evident when nitrite levels were increased in the presence of 0.1 mg/l thiram. In an experimental food chain, Chlorella produced nitrites from nitrates and was toxic via Daphnia to fish (Brachydanio rerio) (25).

Another aspect to be stressed is the risk for environmental species related to the mutagenic potential of thiram itself.

4. CONCLUSIONS

Thiram is embryotoxic in rats at high dose levels and not embryotoxic in rabbits. The dose without effect on the embryos is 7.5 mg/kg in rats and 5 mg/ kg in rabbits (highest dose level). Some indications for teratogenic effects were observed in mice but not in rabbits and rats. Adequate reproduction studies are lacking. In semi-chronic and chronic toxicity feeding studies, effects, especially on body weight, have been found at all dose levels. Furthermore, thiram has demonstrated mutagenic properties in some tests. There are, however, no indications for a carcinogenic effect of thiram by itself. Due to the lack of a no-effect level and the inadequacy of the toxicological data package an ADI cannot be estimated. Therefore, the Committee was unable further to evaluate the safety of the existing Community maximum residue levels (MRLs) at the present time.

Further toxicological data are required in order to assess adequately the safety of thiram. In this context, the following data are particularly necessary: a two generation study in rats, an oral sub-chronic toxicity test in rats showing a no effect level, a dominant lethal test and another *in vivo* mutagenicity test confirming or otherwise the positive results reported by Prasad et al in 1987 (29). In the light of the above considerations, the Committee considered that the current MRLs should be reviewed taking into account current usage patterns with a view to establishing MRLs for individual fruit and vegetables crops, probably at lower levels that the existing figures.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR PESTICIDES ON THE USE OF ALACHLOR AS A HERBICIDE

(Opinion expressed on 25 October 1988)

BACKGROUND AND TERMS OF REFERENCE

Alachlor is the common name for 2-chloro-2', 6'-diethyl-N-methoxymethylacetanilide (BSI, E-ISO, ANSI, WSSA, JMAF). Its solubility in water is 242 mg/l at 25°C and its vapour pressure is 2.9 mPa at 25°C. It is stable to ultra-violet radiation and is hydrolysed under strongly acid or alkaline conditions.

Alachlor is used in certain Member States as a pre-emergence herbicide on maize and also, to a limited exent, as a pre-emergence or post-emergence treatment in soya, sunflower potatoes, green beens, brassicas, etc.

The Commission requested the Scientific Committee for Pesticides to examine the toxicology of alachlor in relation to its application and to give an opinion on the following question: "Is the use, in accordance with good agricultural practice, of alachlor prejudicial to human health or the environment and, if so, can such dangers be eliminated by selective reduction of potential exposure?"

DISCUSSION

1. TOXICOLOGICAL ASPECTS

1.1 Pharmacokinetics in mammals

Comprehensive studies of the pharmacokinetic behaviour of alachlor revealed that no significant differences in the metabolism exist which are dependent on the route of administration or on the doses applied. However, there are considerable differences in metabolism, distribution and excretion, between rodents and primates. Extensive studies were performed in rats and monkeys but those for the mouse were limited and inadequate. Some metabolic studies in operators were also available (1-16, 22, 23).

The excretion of alachlor and its metabolites in urine and faeces is much slower in rats (biphasic: b- half life of approximately 7 days) than in mice (monophasic: half life of approximately 24 hours); whilst in monkeys the overall elimination half-lives in a two-compartment model were calculated at 3.5 and 6.5 hours (12, 14-21).
Mice excrete alachlor metabolites predominantly via the faeces; rats 50:50 - urine: faeces; whilst in monkeys excretion via urine prevails (12, 14-16, 22, 23).

It is assumed that a major group of the numerous metabolites in the rat urine (14 identified out of 30, e.g., the methylsulfoxides and methylsulfones) are formed by biliary excretion of mercapturic acid pathway metabolites with their subsequent enterohepatic circulation and extensive further metabolism. A similar complex pattern of urine metabolites was observed in mice but with large quantitative differences.

In monkeys, only five major urinary metabolites were found and identified. In contrast with rats and mice, metabolism in monkeys mainly occurs by conjugation with glutathione, followed by transformation via the mercapturic acid pathway, leading to a mercapturic acid and a cysteine conjugate. Furthermore, methylsulfoxide and methylsulfone metabolites were not found in monkeys' urine, indicating that enterohepatic circulation does not make a significant contribution to the metabolism of alachlor in monkeys (12, 14-17, 19, 21, 23-32).

A suspected key metabolite with respect to the carcinogenicity of alachlor is 4-amino-3,5-diethylphenol (ADEP). It was found in much larger quantities in the urine of rats than in mice and monkeys. In vitro studies with liver and nasal tissue preparations showed that 2,6-diethylaniline (DEA) is a likely precursor for ADEP, which is excreted as the sulfate conjugate. In rats both tissues contain enzymes of high activity necessary to convert secondary amide metabolites of alachlor to DEA and to oxidise DEA to ADEP. This phenol intermediate may give rise to the formation of a highly electrophilic benzoiminoquinone, which is thought to be the ultimate carcinogenic alachlor metabolite. Oral administration of ^{14}C labelled alachlor resulted in high levels of radioactivity in rat nasal turbinates but not in mice and monkeys. Application of a main rat metabolite, alachlor-methylsulfide, resulted in an even higher accumulation in rats (4, 14-18, 20, 23, 26-30, 33, 34).

1.2 Acute/short term toxicity

Alachlor is of low to moderate acute oral toxicity with LD_{50}-values in rats of 903 to 1350 mg/kg body weight (b.w.). It is an irritant to skin and mucous membranes and induces skin sensitization in guinea pigs and hyperreactive humans (35-44, 50).

In rather poor 90-day feeding studies in rats and dogs a no-effect-level (NOEL) of 200 mg/kg feed (10 mg/kg b.w./day) was claimed for rats. In dogs a NOEL was not derived because of small group sizes and other shortcomings. In a 6-month gavage study in dogs, even the lowest dose (5 mg/kg b.w./day) still caused changes in liver weight. A NOEL could not be established (45-48).

A 1-year gavage study in dogs resulted, at the highest dose level (10 mg/kg b.w./day), in reduced body weight gain, changes in liver weight, testicular atrophy, hematology, and clinical chemistry. Doses of 3 mg/kg b.w./day and above caused hemosiderin deposition in liver, kidneys, and spleen. A NOEL of 1 mg/kg b.w./day (lowest dose) can be assumed (49).

1.3 Effects on reproduction

Two teratological studies in rabbits gave no indications of teratogenic or foetotoxic effects at maternal nontoxic dosages up to 60 mg/kg b.w./day (43, 79, 132).

In pregnant rats, dosages of 50 mg/kg b.w./day and above during the critical stages of gestation caused maternal toxicity. At 150 mg/kg b.w./day foetal weights were reduced and resorptions increased (54).

A three-generation feeding study in rats revealed, even at the highest dose (equivalent to 30 mg/kg b.w./day), no significant changes except for reduced ovarian weights in all generations (43, 55).

1.4 Long-term toxicity

An 18-month feeding study in mice with dosages equivalent to 26, 78 and 260 mg/kg b.w./day showed a high incidence of

amyloidosis in all groups including the controls. Dose-related increases in liver weight were observed at the mid and high-dose levels. Retinal atrophy and degeneration were reported (5% controls; 8% low dose; 8% mid dose; 12% high dose). A NOEL cannot be derived from this study (43, 56, 59, 61).

Three long-term studies were carried out in rats. In the first 2-year feeding study in Long-Evans rats, with dietary levels equivalent to 14, 42 and 126 mg/kg b.w./day, ophthalmoscopy revealed a dose-related uveal degeneration. A NOEL could not be established because of a 5% incidence of uveal degeneration at the lowest dose level (43, 57).

In the second study in Long-Evans rats, with a dose level equivalent to 126 mg/kg b.w./day for up to 2 years, the ocular lesions were investigated in detail. The results of the first study were confirmed and the lesions shown to be irreversible (62).

In the third 25-month study in Long-Evans rats lower dietary alachlor levels, equivalent to 0.5, 2.5, and 15.0 mg/kg b.w./day were used. Mortality rates in the control and all treatment groups reached 50% at the termination of the study. Ocular lesions were not observed. A NOEL of 2,5 mg/kg b.w./day for non carcinogenic toxic effects can be derived (58).

1.5 Mutagenicity

Numerous mutagenicity studies *in vitro* (with and without activation) and *in vivo* with alachlor gave negative results in conventional tests. However, *in vivo* and *in vitro* rat hepatocyte DNA repair assays were weakly positive. Furthermore, some tests with plant systems were positive (3, 23, 63-66, 68-73, 78, 83-93, 107).

Whereas, bile from alachlor treated rats was negative in the Ames-Test with the usual Salmonella typhimurium strains, the urine produced weak mutagenic responses in TA98 and TA1537 in the presence of b -glucuronidase. Furthermore, three rat urinary metabolites, clearly not connected with the proposed pathway to the suspected carcinogenic benzoiminoquinone, proved positive in TA98. There are positive mutagenicity data showing that DEA is positive. Mutagenicity data on the proposed key metabolite ADEP are unavailable (3, 23, 67, 68, 75-77, 79-82, 98, 134).

1.6 Carcinogenicity

The 18-month chronic/carcinogenicity study in mice (dietary levels equivalent to 26, 78 and 260 mg/kg b.w./day) showed a dose-related trend of increased incidence of lung bronchiolar-alveolar neoplasms in females significant at the highest dose level and increased hepatocellular tumours in males significant at only the highest dose level. Animals sacrificed at the termination of the study revealed that the total number of tumours and the number of tumour bearing mice increased dose dependently. In mice that died or were sacrificed during the study the percentages of tumour bearing mice and of total tumours were enhanced at the high dose but reduced at the mid-dose levels. High mortality (> 50%) was observed in this study in all groups (including controls) due probably to a high incidence of anmyloidosis in many of the test animals. Since only lung and liver tumours were found in mice which are common in this species, and despite the shortcomings of this study there is not sufficient evidence to conclude that alachlor is carcinogenic in mice (29, 56, 59, 61).

The first chronic/carcinogenic 2-year study in rats was carried out with feed levels equivalent to 14, 42 and 126 mg/kg b.w./day over two years. Nasal turbinate tumours (mainly adenoma) were dose-related in the mid and high-dose groups and in each of these, three ependymomas (brain tumours) were found showing pathological signs, indicating metastases of nasal adenocarcinomas. In addition, the incidence of malignant stomach tumours and thyroid follicular tumours was increased at the highest dose level. From the weight loss reported, it may be concluded that the highest dose level exceeded the maximum tolerated dose (MTD) (43, 57, 94).

The second chronic/carcinogenic 2-year rat study with dose levels equivalent to 0.5, 2.5 and 15.0 mg/kg b.w./day revealed an increased incidence of nasal epithelial adenoma at the highest dose level. However, an increase in the incidence of stomach, thyroid and brain tumours was not found (58).

In the third chronic rat study, not designed as a carcinogenic study, a single dose level was used (equivalent to 126 mg/kg b.w./day for up to two years). In one subgroup which received alachlor for 5 to 5.5 months, an increased incidence of nasal turbinate adenomas in both sexes was observed at study termination. Another subgroup which received alachlor during the whole study period showed, in addition, elevated incidences of adenocarcinomas of the nasal turbinate, malignant tumours of the stomach (carcinoma/sarcoma) and neoplasms of the thyroid (follicular adenoma + carcinoma, C-cell adenoma) (58, 62).

1.7 Human data - occupational exposure

Dermal deposition data for alachlor operators showed considerable variations independent of formulation used or operator function. The possible exposure of the spray operator (with an 80% protection) using 4.4 kg alachlor active ingredient (A.I.)/ha (emulsifiable concentrate (EC) was calculated to be in the order of 2×10^{-2} mg/kg/day on the day of application. The rate of application was nearly double the generally recommended dosage. Such exposure should result in a life-time average exposure of 3×10^{-5} mg/kg/day or 1×10^{-3} mg/kg/day, assuming alachlor application on 1 or 30 days per year respectively. Exposure using other formulations would be slightly less; for example, the possible exposure of a spray operator on the day of application using micro-encapsulated alachlor at the rate 4.4 kg a.i./ha was calculated to be 9×10^{-3} mg/kg/day (53, 96, 97).

Urinary excretion of alachlor and its metabolites containing DEA and HEEA moieties ranged between 0 and 132 ug/person/application day (mean 41.5 ug) per kg alachlor applied. As a result of the low excretion level, a detailed characterisation of the metabolites was not possible (53, 95).

The calculated dermal absorption levels ranged from 0.37 to 11.4% for micro-encapsulated alachlor and from 12.5 to 25% for the emulsifiable concentrate preparation (96, 97, 130, 133).

Vital status follow-up of plant workers exposed to alachlor for up to 12 years gave no indications that cancer deaths were related to exposure. Ocular lesions were no more evident in the exposed than in the control individuals (60, 99).

2. FATE IN PLANTS

The metabolism of alachlor in plants is well understood. The parent compound and some of its microbial soil metabolites may be taken up from treated soils and translocated in plants.

The main metabolic pathways appear to be via:

- complete displacement of the alachlor chlorine moiety by oxygen or sulphur nucleophiles;
- hydroxylation at the benzyl position;
- sugar conjugation.

Considerable quantitative differences between plant species were found. Residue determination should include the parent compound and metabolites containing the diethylaniline (DEA) and hydroxyethyl-ethylaniline (HEEA) moieties. Residues resulting from registered uses can be expected generally not to exceed 0.21 mg/kg in soybeans and 0.016 mg/kg in maize. There is no evidence for the production of the suspected animal carcinogens DEA and ADEP in plants (1, 3, 100-103).

3. FATE IN DOMESTIC ANIMALS

Studies by oral administration of ^{14}C labelled alachlor to laying-hens and goats showed that the major part of alachlor and its metabolites was excreted rapidly. In hens about 90% was eliminated at the end of the dosing period (6 days) and only 0,07% of the administered radioactivty was detected in eggs, 0,03% in livers and 0,02% in other tissues (51, 52).

In lactating goats dosed for 5 days, 81% of the administered dose was excreted rapidly via urine and faeces (42,3 and 30,7%

respectively), whilst milk and each of the tissues analysed (muscle, liver, kidney and fat) contained, at the end of the dosing period, less than 0,5% of the administered radioactivity (104, 105).

Based on these studies it may be concluded that with current usage patterns in the Community, no measurable amounts of alachlor or its metabolites may be expected in meat, milk or eggs when treated crops or their wastes are fed at normal rations to dairy animals and poultry.

4. FATE AND EFFECTS IN THE ENVIRONMENT

4.1 Soil

Degradation of alachlor in soils is due predominantly to bacterial and fungal metabolism. It leads to a mixture of oxanilic and sulfonic acids and to benzylic hydroxylation and formation of diethylaniline (DEA), respectively. DEA interacts rapidly with humic substances in the soil (106, 108-111, 112, 113, 121, 139).

Biotic degradation under aerobic conditions showed half-life values between 6 and 25 days dependent on the soil type (108-110, 121). No build up of parent alachlor in the soil following applications at the recommended use rates should be expected.

Application of alachlor to sandy soils poses a risk of leaching (106, 121). During leaching extensive metabolic degradation mainly to oxanilic, sulfonic and sulfonylacetic conjugates takes place (108, 116, 121).

Considerable volatilization can be expected especially in moist soils, at higher temperatures and in windy conditions, leading possibly to a seasonal contamination of precipitation (114, 115, 121, 138).

4.2 Water

Occasional contamination of groundwater has been observed with alachlor levels of up to 16.6 ug/l (53, 117, 119, 136). Degradation of alachlor itself in groundwater and the mineralization in surface water are apparently slow (116, 117).

Municipal water supplies from rivers in areas with high usage of alachlor showed contamination up to 100 ug/l due to run-off (128). In tap water, concentrations between 0.22 and 5.1 ug alachlor/l were sometimes reported following conventional surface water treatment without carbon filtration.
In a monitoring study, conducted by Monsanto (135), little if any difference was seen between corresponding raw and finished water from 24 community water systems. In this study maximum weekly concentrations in finished water were detected up to 10.9 ug alachlor/1 for the growing season. In a later monitoring study up to 9.48 ug alachlor/1 was found in finished water (137). Data on degradation products of alachlor in raw and drinking water were not available (117, 118, 120, 121, 131, 135- 137).

4.3 Ecotoxic effects

The acute toxicity of alachlor on aquatic indicator organisms is low as judged by the effects of alachlor levels in surface water and even from cases of accidental

spillages. This suggests that the use of alachlor under normal conditions does not present environmental risks to aquatic life. Available data indicates that acute toxicity to the fauna of the agroecosystem is low. Furthermore, bioaccumulation is unimportant. However, long-term experimental data are not available for aquatic or agroecosystem flora and fauna (115, 122 - 129).

5. CONCLUSIONS

Studies with respect to non-carcinogenic toxic effects of alachlor revealed:

a) low to moderate acute oral toxicity, with irritating and sensitizing effects;

b) subchronic and chronic toxic effects in mice, rats and dogs, comprising increased liver weight and species dependent pathological changes in other organs. Special noteworthy findings are testicular atrophy in dogs, retinal atrophy and degeneration in mice and uveal degeneration in rats. It can be assumed that the NOEL for non-neoplastic effects is 1 mg/kg b.w./day in dogs and 2,5 mg/kg b.w./day in rats.

The studies with respect to teratogenic, mutagenic and carcinogenic effects of alachlor showed:

a) There are no indications that alachlor affects reproductive functions or causes terata in pups;

b) Alachlor itself is not mutagenic, although its metabolite DEA has been shown to be mutagenic.

c) Alachlor is clearly carcinogenic in rats causing mainly nasal turbinate tumours. Its carcinogenic effects are thought to be related to its metabolites, especially DEA, ADEP and benzolminoquinone.

d) There is not sufficient evidence that alachlor is carcinogenic in mice. However, in this context it must be noted that the mouse study shows certain inadequacies. Therefore, species specificity for rats cannot be proven.

Data on metabolites in laboratory animals show that there are considerable differences in metabolism between rats, mice and monkeys. The possible carcinogenic metabolites are mainly formed in rats but also in low amounts in mice and monkeys. Furthermore, it appears from autoradiographic studies that alachlor derived material in rats is concentrated in the nasal turbinates - this is not found in either mice or monkeys.

The species differences in the pharmacokinetics of alachlor give a reasonable explanation for the high susceptibility of rats to the carcinogenic effects of alachlor. However, they cannot exclude its carcinogenicity to other species, e.g. mice, monkeys and humans. It has to be assumed that the species differences are quantitative rather than qualitative.

The metabolism of alachlor in plants is well understood. The parent compound and some of its soil metabolites may be taken up from treated soils and are translocated through the entire plant. Residue data for alachlor (including its metabolites containing DEA and HEAA moieties) is desirable for food crops for which alachlor is authorised in the Community (eg beans, potatoes). In view of the current usage pattern in the Community, dietary exposure from plant sources may be considered low.

No measurable amounts of alachlor or its metabolites may be expected in meat, milk or eggs, following recommended pre-emergence use of alachlor on crops fed to domestic animals. Therefore, dietary exposure is low from these sources.

Since exposure of the general population, via food of plant and animal origin and drinking water is low, the margin of safety with respect to the suspected carcinogenicity for the general population is currently regarded as sufficient.

In comparison with the general population, operators are exposed to a greater extent to alachlor. However, considering the demonstrated species quantitative differences and provided that precautions are taken to minimize exposure, the margin of safety for operators with respect to the suspected carcinogenicity is regarded as sufficient.

Application of alachlor to sandy soils poses a risk of leaching and thereby also of contamination of groundwater. Contamination of surface waters is mostly due to run-off from treated soils. Therefore, appropriate precautions should be taken to minimize run-off of alachlor and its use in soil conditions favourable to leaching should be avoided.

Data on degradation of alachlor in raw water are required.

Toxic effects of alachlor to aquatic and terresterial fauna are not likely to occur under normal conditions of use.

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