

METHIOCARB

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Explanation

Methiocarb was evaluated for toxic effects by Joint Meetings in 1981, 1983, 1984, 1985, and 1987 (Annex 1, references 36, 40, 42, 44, and 50). An ADI of 0–0.001 mg/kg bw was allocated in 1981, which was extended at subsequent meetings. Methiocarb was evaluated by the present Meeting within the CCPR periodic review programme. This monograph summarizes the new data and relevant data from the previous monographs and monograph addenda on methiocarb (Annex 1, references 37, 41, and 46).

Evaluation for Acceptable Daily Intake**1. Biochemical aspects***(a) Absorption, distribution, and excretion*

[ring-1-¹⁴C]-Methiocarb (4.8 mCi/mmol; purity, > 97%) was administered to three female rats by gavage at a dose of 20 mg/kg bw. Similarly labelled methiocarb (14.5 mCi/mmol; purity, > 97%) was administered to three male and three female rats by gavage at a dose of 0.25 mg/kg bw. Organic extracts of 48-h urine samples collected from each rat were subjected to one-dimensional or two-dimensional thin-layer chromatography on plates pre-coated with silica gel. More than 90% of the radiolabel administered at the higher dose was excreted in the urine. Similar results were obtained with the lower dose, but a somewhat smaller proportion of the radiolabel

(73–86%) was excreted over 48 h; at this dose, there was no major difference between males and females (Stanley & Johnson, 1976).

(b) *Biotransformation*

In the urine samples collected by Stanley and Johnson (1976), the major components of the chloroform extract at the higher dose were methiocarb phenol, methiocarb sulfoxide phenol, and an unknown component which may correspond to *N*-hydroxymethyl methiocarb sulfoxide. A trace of methiocarb sulfoxide was detected. After incubation of the aqueous phase of the urine samples with maltase, about half of the radiolabel present was rendered organosoluble, the major component being methiocarb sulfoxide phenol; methiocarb phenol and methiocarb sulfone phenol were also present. The results were similar at the lower dose, at which there was no major difference between males and females; however, methiocarb sulfone phenol was found in the chloroform extract.

These results differ from those of an earlier study in rats with [carbonyl-¹⁴C]- and [methyl-³H]-methiocarb, which was not available for evaluation. It was reported that methiocarb phenol was the primary metabolite in the earlier study, whereas in the study of Stanley and Johnson (1976), methiocarb sulfoxide phenol was the metabolite at highest concentration in the urine. The report of the latter study also cites an earlier study in dogs in which the only metabolites seen were methiocarb sulfoxide phenol and methiocarb sulfone phenol.

In studies of the metabolism of methiocarb *in vitro* (Strother, 1970, 1972; Menzie, 1974) with ¹⁴C-methiocarb, human liver fractions were slightly less metabolically active than fractions from Sprague-Dawley rats. The major metabolite was methiocarb sulfoxide. In a comparative study of the metabolism of methiocarb (and Zectran, a related compound) in rat and dog liver and kidney homogenates and various blood fractions *in vitro*, the metabolic pathway seemed to be similar in the two species, sulfoxidation of the sulfur atom being the main route. *N*-Methyl hydroxylation was also found to occur (Wheeler & Strother, 1971).

Flavin adenine dinucleotide-dependent monooxygenases from pig liver microsomes had some activity in the sulfoxidation of methiocarb (Haijar & Hodgson, 1982). Methiocarb is reported to cross the placenta into the fetus (Salama et al., 1993).

The main products of metabolism of methiocarb in plants were conjugates of the phenol, sulfoxide phenol, and sulfone phenol; methiocarb sulfone was also seen, while methiocarb sulfoxide occurred in some plant products (Murphy et al., 1982).

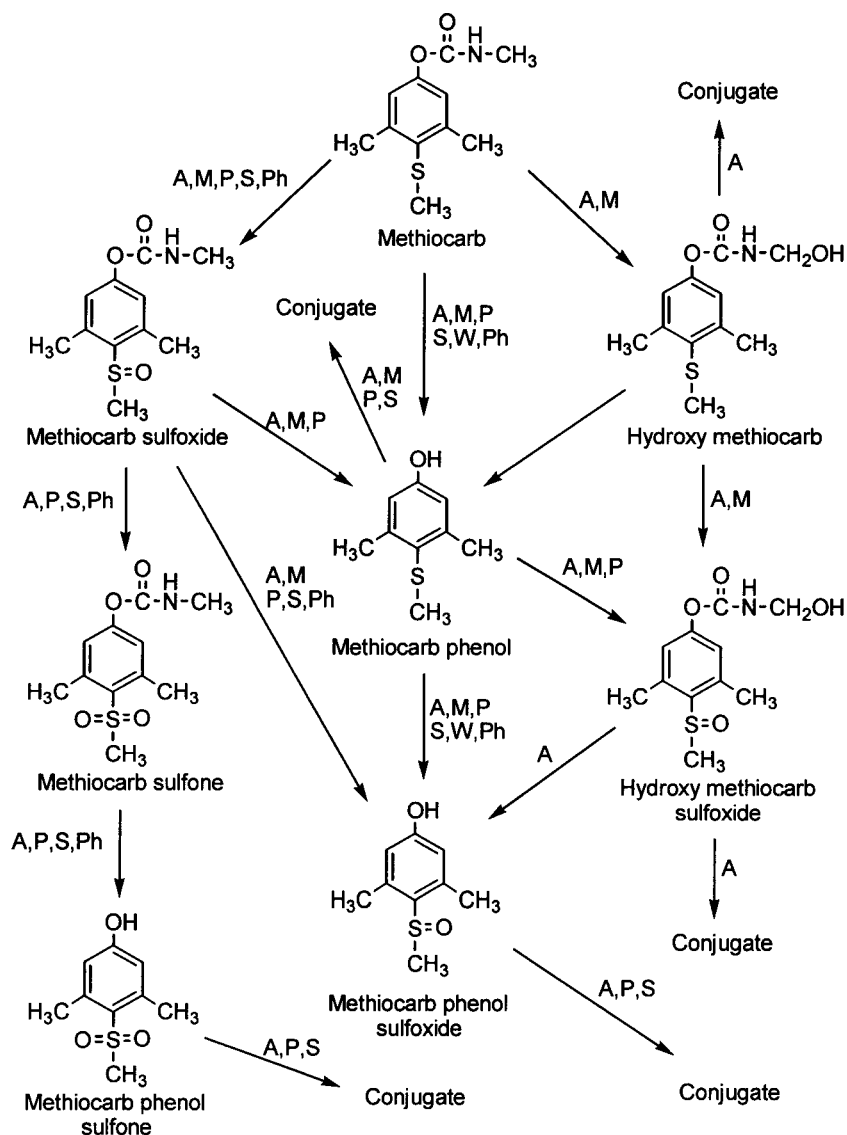
Figure 1 shows the proposed metabolic pathways for methiocarb in various species and media.

(c) *Effects on enzymes and other biochemical parameters*

Single doses of 1, 10, 25, or 50 mg/kg bw methiocarb were administered by gavage to groups of five Wistar rats of each sex, while five rats of each sex acted as controls. Plasma and erythrocyte cholinesterase activity was measured after 20 min, 2 h, and 5 h, and, in males at the highest dose, additionally at 1.5 and 3 h; brain acetylcholinesterase activity was measured at 30-min intervals up to 5 h. Cholinergic signs, starting immediately after treatment and abating within 2 h, were seen at ≥ 10 mg/kg bw. Male rats receiving the highest dose died after 2–3 h. Maximum depression of plasma cholinesterase and erythrocyte acetylcholinesterase activity was observed after 20 min at ≤ 25 mg/kg bw and after 20 min to 2 h at the highest dose. In a separate study, methiocarb was given at a dose of 10 or 20 mg/kg bw, and brain acetylcholinesterase activity was determined at 30 min and 1, 2, 3, and 5 h. Inhibition was maximal at 2 h.

In a four-week study, 10 rats of each sex received methiocarb at doses of 1, 3, or 10 mg/kg bw per day by gavage. Plasma and erythrocyte cholinesterase activity was determined in three rats of each sex 20 min after administration of the test material on days 4, 8, 14, 21, and 28; brain acetylcholinesterase activity was determined in five rats of each sex 2 h after the last dose. At the

Figure 1. Proposed metabolic and reaction pathways of methiocarb in animals (A), microorganisms (M), plants (P), soil (S), and water (W) and during photodegradation (Ph)



highest dose, cholinergic signs were observed briefly. Plasma, erythrocyte, and brain cholinesterase activity was depressed at 10 mg/kg bw per day. The NOAEL for cholinesterase depression was thus 3 mg/kg bw per day (Eben & Kimmerle, 1973).

Technical-grade methiocarb (purity, 97%) and methiocarb sulfoxide (purity, 95.2%) were administered by gavage to groups of 15 female Sprague-Dawley-derived rats at a dose of 0.5 or 2 mg/kg bw per day on five days per week for four weeks, while 15 controls received the vehicle (Carbowax). The rats were observed daily for general appearance and, in addition, for cholinergic signs at 0.5, 1, and 4 h after dosing for the first five days of treatment. For determination of cholinesterase activity, each group was subdivided into three subgroups of five. Blood was collected from the first subgroup before and 30 min after dosing on days 0, 7, 14, 21, and 28; blood was collected similarly from the second group but 4 h after dosing on days 4, 11, 18, and 25. The third subgroup was held in reserve in case anaemia developed. Depression of cholinesterase activity was calculated by comparison with activity before treatment.

Tremors were seen during the first five days of the study in some rats receiving the sulfoxide at the higher dose; no clinical signs were seen in other groups. A 20% depression in plasma cholinesterase activity was found in controls 4 h after dosing on day 25, and depressions of 1–21% were found 30 min after dosing with methiocarb at 0.5 mg/kg bw; 4 h after dosing at 0.5 mg/kg bw, depressions of < 10% were noted. At the higher dose of methiocarb, depressions of 19–41% were seen 30 min after dosing and 0.2–19% 4 h after dosing. With the sulfoxide, plasma cholinesterase activity was inhibited by 21–39% at the low dose at 30 min but was generally not inhibited at 4 h. At the high dose, 39–62% inhibition was seen at 0.5 h and 10–25% inhibition at 4 h. Erythrocyte acetylcholinesterase activity was depressed by 5–12% 30 min after dosing at 0.5 mg/kg bw and by < 10% 4 h after dosing. At the higher dose of methiocarb, erythrocyte acetylcholinesterase activity was depressed by 15–29% 30 min after dosing and by 1–13% 4 h after dosing. With the sulfoxide, erythrocyte acetylcholinesterase activity was inhibited by 13–31% at the low dose at 30 min but was generally not inhibited at 4 h. At the high dose, 32–46% inhibition was seen at 0.5 h and 10–21% at 4 h. The NOAEL for erythrocyte acetylcholinesterase inhibition was therefore approximately 0.5 mg/kg bw per day for methiocarb, but there was no NOAEL for the sulfoxide (Hixson, 1981).

Technical-grade methiocarb (purity, 97%) and methiocarb sulfoxide (purity, 95.2%) were administered orally in gelatine capsules to groups of two adult beagle dogs and two bitches at a dose of 0.05 or 0.5 mg/kg bw per day for 29 days; two control animals of each sex received empty capsules. The animals were observed twice daily, and blood was taken for measurement of plasma and erythrocyte cholinesterase activity before the first dose, 1, 3, 6, and 24 h after the first dose, and 3 h after the third dose. During the second week, blood was taken before the first dose, 2, 6, and 24 h later, and 2 h after the second dose. During weeks 3 and 4, blood was taken before the first dose, 2, 6, and 24 h later, and 2 h after the third dose. In week 5, blood was again taken before the first dose and 2, 6, and 24 h later; dosing was then stopped, and another blood sample was taken on the third day.

Clinical signs of toxicity (salivation and vomiting) were observed at the higher dose of each test material and in animals of each sex; additionally, slight salivation was observed in one bitch given the sulfoxide at the lower dose. Depression of cholinesterase activity was calculated by comparison with the level measured just before the first dose each week: inhibition was variable, but peak inhibition occurred 0–3 h after dosing; considerable inhibition was seen with both materials at the higher dose in animals of each sex. At the lower doses, smaller depressions of both plasma and erythrocyte cholinesterase activity, of up to about 20%, were sometimes seen with both materials. Cholinesterase activity was generally normal by 6 h (Hayes, 1981).

At equimolar concentrations, methiocarb was the least effective of four carbamates in inhibiting bovine erythrocyte acetylcholinesterase and equine plasma cholinesterase activity (Barthová et al., 1989).

Methiocarb was reported not affect liver function in rabbits at a single oral dose of 25 mg/kg bw (Kimmerle, 1960).

2. Toxicological studies

(a) Acute toxicity

The results of studies of the acute toxicity of methiocarb and putative metabolites of methiocarb are shown in Table 1.

No erythema or oedema was seen when technical-grade methiocarb (purity unspecified) was applied to abraded and unabraded skin of six New Zealand white rabbits. Application to the eyes of six rabbits did not produce ocular irritation (Crawford & Anderson, 1970).

Table 1. Acute toxicity of methiocarb and putative metabolites

Species	Strain	Sex	Route	LD ₅₀ (95% CI or range) (mg/kg bw)	Purity (%)	Reference
Methiocarb						
Rats	Sprague-Dawley	M	Oral	130	Technical	DuBois & Raymund (1962)
		F		140		
Rats	Sprague-Dawley	F	Oral	100	Recrystallized	DuBois & Raymund (1962)
Rats	NR	NR	Oral	67	NR	Kimmerle (1966a)
Rats	Sprague-Dawley	M	Oral	30 (20-45)	99	Crawford & Anderson (1973)
		F		30 (20-45)		
Rats	Sprague-Dawley	M	Oral (non-fasting)	46 (38-56)	99	Crawford & Anderson (1973)
		F		47 (36-63)		
Rats	Sprague-Dawley	M	Oral	15 (9-26)	Technical	Lamb & Matzkanin (1976a)
		F		31 (18-54)		
Rats	Sprague-Dawley	M	Oral	13 (9-17)	Technical	Lamb & Matzkanin (1976b)
		F		32 (24-44)		
Rats	Sprague-Dawley	M	Oral	14 (12-16)	Technical	Lamb & Matzkanin (1977)
		F		16 (13-20)		
Rats	Sprague-Dawley	M	Oral (non-	51 (45-58)	Technical	Lamb & Matzkanin (1977)
		F		79 (65-96)		
Rats	NR	M	Oral	22 (19-25)	98.5	Flucke (1978)
		F		24 (21-28)		
Rats	NR	M	Oral	22.1 (18-27)	98.3	Flucke (1980)
Rats	Sprague-Dawley-	M	Oral	33 (22-50)	98	Nelson (1979)
	derived	F		47 (31-70)		
Rats	NR	M	Oral	17 (16-19)	98.6	Heimann (1983)
Rats	NR	M	Oral	19 (16-23)	98.2	Flucke (1988)
		F		26 (19-36)		
Rats	Albino	M	Oral	100	NR	Kimmerle (1960)
Rats	Wistar-CFN	M	Oral	87	NR	Klimmer (1963)
Rats	NR	M	Oral	33 (29-38)	98.4	Thyssen (1977a)
				35 (29-42)	98.3	
				35 (30-42)	98.2	
				31 (26-35)	97.8	
				28 (24-33)	97.4	
Rats	Sprague-Dawley	M	Intraperitoneal	35	Technical	DuBois & Raymund (1961a)
		F		30		
Rats	Sprague-Dawley	F	Intraperitoneal	25	Recrystallized	DuBois & Raymund (1962)
Rats	Wistar-CFN	M	Intraperitoneal	43	NR	Klimmer (1963)
Rats	Sprague-Dawley	M	Dermal	> 200	Technical	DuBois & Raymund (1961a)
Rats	Sprague-Dawley	F	Dermal	> 300	Recrystallized	DuBois & Raymund (1962)
Rats	Albino	M	Dermal	> 1000	NR	Kimmerle (1960)
Rats	Wistar-CFN	M	Dermal	350-400	NR	Klimmer (1963)
Rats	NR	M	Dermal	> 500	99.2	Solmecke (1969)
Rats	Wistar	M	Dermal	> 5 g	98.1	Thyssen (1977b)
		F		> 5		
Rats	Sprague-Dawley	M	Inhalation (1 h)	1200 mg/m ³ ^a (780-1700)	98.8	Shiotsuka (1987a)
		F		1100 mg/m ³ ^a (740-1600)		
Rats	NR	M	Inhalation	540 g/L ³	NR	Kimmerle (1966b)
Rats	Sprague-Dawley	M	Inhalation (1 h)	> 20 000 g/L	Technical	Crawford & Anderson (1972a)
		F		> 20 000 g/L		
Rats	Sprague-Dawley	M	Inhalation (4 h)	580 mg/m ³ (340-700)	98.8	Shiotsuka (1987b)
		F		430 mg/m ³ (290-580)		
Rats	Wistar	M	Inhalation (4 h)	> 320 mg/m ³	97.9	Thyssen (1982)
		F		> 320 mg/m ³		
Rats	Wistar	M	Inhalation	> 300 mg/m ³	97.9	Thyssen (1982)
		F	(5 x 6 h)	> 300 mg/m ³		
Mice	NR	M	Oral	25 (21-30)	NR	Kohgo (1970)
Mice	NR	M	Oral	52	98.2	Kimmerle (1972)
Mice	NR	M	Subcutaneous	940 (760-1200)	NR	Kohgo (1970)
Mice	Carworth Farm	M	Intraperitoneal	6	Technical	DuBois & Raymund (1961a)
		F		5.5		
Mice	Dierolf Farm	F	Intraperitoneal	16	Technical	Baron et al. (1964)
Rabbits	New Zealand white	M	Dermal	> 2000	99	Crawford & Anderson (1972b)
		F		> 2000		
Guinea-pigs	NR	M	Oral	40	Technical	DuBois & Raymund (1961a)
Guinea-pigs	NR	F	Oral	(50-100)	99.2	Kimmerle (1969a)
Guinea-pigs	Albino	F	Oral	14 (7.9-25)	Technical	Crawford & Anderson (1972a)
Guinea-pigs	NR	M	Intraperitoneal	17	Technical	DuBois & Raymund (1961a)
Dogs	Beagle	F	Oral	(10-25)	99.2	Kimmerle (1969b)
Dog	Mongrel	M	Oral	~ 25	Technical	Lamb & Matzkanin (1975)
		F		~ 25		

Table 1 (contd)

Species	Strain	Sex	Route	LD ₅₀ (95% CI or range (mg/kg bw)	Purity (%)	Reference
Chicken	NR	F	Oral	175	Technical	DuBois (1962)
Chicken	White Leghorn	F	Oral	380 (300–490)	98.5	Thyssen & Schilde (1978)
Methiocarb phenol						
Rats	NR	M	Oral	> 1000	NR	DuBois (1964)
Rats	NR	M	Dermal	> 1000	NR	DuBois (1964)
Rats	NR	NR	Oral	> 1000	NR	Solmecke (1970)
Methiocarb phenol sulfoxide						
Rats	NR	M	Oral	> 1000	NR	DuBois (1964)
Rats	NR	NR	Oral	> 1000	NR	Solmecke (1970)
Rats	NR	M	Dermal	> 1000	NR	DuBois (1964)
Methiocarb phenol sulfone						
Rats	NR	M	Oral	> 1000	NR	DuBois (1964)
Rats	NR	NR	Oral	> 1000	NR	Solmecke (1970)
Rats	NR	M	Dermal	> 1000	NR	DuBois (1964)
Methiocarb sulfoxide						
Rats	NR	NR	Oral	43 (37–50)	NR	Solmecke (1970)
Rats	Sprague-Dawley	M	Oral	9 (7–13)	NR	Lamb & Matzkanin (1976a)
		F		7		
Rats	Sprague-Dawley	M	Oral	6 (5–8)	NR	Lamb & Matzkanin (1976a)
		F		8 (6–10)		
Methiocarb sulfone						
Rats	NR	NR	Oral	> 1000	NR	Solmecke (1970)
Hydroxymethyl methiocarb						
Rats	NR	M	Oral	> 110	NR	Nelson (1979)
		F		> 110		
Hydroxymethyl methiocarb sulfone						
Rats	NR	M	Oral	> 110	NR	Nelson (1979)
		F		> 110		
Hydroxymethyl methiocarb sulfoxide						
Rats	NR	M	Oral	> 160	NR	Nelson (1979)
		F		> 160		

Technical, purity unstated; NR, not reported

^a There was a large discrepancy between nominal and measured concentrations owing to deposition in the chamber; these figures were measured gravimetrically.

A study of the skin-sensitizing potential of methiocarb (purity, 97.8% pure) was conducted by the Magnusson and Kligman technique. A group of 20 guinea-pigs (strain BOR:DHPW) each received methiocarb intradermally at a concentration of 1% and topically at a concentration of 25%. There were two control groups of 10 guinea-pigs. The test animals were first challenged with methiocarb at a concentration of 25% and then at 12.5%. Although there was a small excess in the number of animals that reacted positively after the second challenge, the overall result was considered to be negative (Mihail, 1984).

In a test for dermal sensitization by the Buehler technique, technical-grade methiocarb (purity, 99.2%) was applied to 15 Hartley guinea-pigs once weekly for three weeks, with a challenge dose a fortnight later. The material was applied as a single dose to five previously unexposed animals at the time of the challenge dose. Dinitrochlorobenzene, used as the positive control, was applied in the same way to a further group of five guinea-pigs, weekly for three weeks, with a challenge dose two weeks later, and to a further group of five as a single dose at the time of the challenge dose. Methiocarb was not considered to be a dermal sensitizer (David, 1988).

A case of allergic contact dermatitis was reported in a man who grew carnations. He developed acute severe eczema of the hand and showed a positive reaction in a patch test to 0.5% methiocarb (Willems et al., 1997).

*(b) Short-term studies of toxicity**Rats*

Methiocarb in aqueous tragacanth suspension was given by gavage to albino rats daily. The dose was 2 mg/kg bw per day for the first three days and 4 mg/kg bw per day for the next 24 days. Two groups of three animals were killed every week for determination of cholinesterase activity. The activity had fallen to 80% of the control values after 14 days and to 50% by the end of the study. No abnormal clinical signs were observed; the animals gained weight normally (Kimmerle, 1960).

Methiocarb was administered by gavage to groups of 25 male Wistar-CFN rats at a dose of 2.5 or 5 mg/day on six days per week for six months. Three deaths occurred at the higher dose due to bronchopneumonia, but weight gain was the same in the two treated groups and in 20 control animals; no abnormal clinical signs were seen (Klimmer, 1963).

Groups of 12 Sprague-Dawley rats of each sex were fed diets containing methiocarb (purity unspecified) at concentrations of 0, 5, 10, or 50 ppm for 16 weeks. Five animals of each sex from each group were examined histopathologically, and five of each sex were used for measurements of cholinesterase activity in blood, brain, and submaxillary glands by a manometric method. No effect on growth rate, food intake, or mortality rates was observed at any dose. In males at the highest dose, serum, erythrocyte, brain, and submaxillary gland cholinesterase activity was inhibited by 21, 14, 12, and 7%, respectively. In females at the highest dose, serum cholinesterase activity was reduced by 28% and erythrocyte acetylcholinesterase activity by 15%, all by comparison with concurrent controls. Brain acetylcholinesterase activity was reduced by 5% in females, and inhibition of submaxillary gland enzyme was seen in all test groups. A NOAEL could not be identified in this study (Doull et al., 1962).

Groups of 10 Wistar rats of each sex were exposed to an aerosol of methiocarb (purity, 97.9%) of a mass median diameter of 2.1–2.5. The groups were exposed to either air, solvent, methiocarb at 6 mg/m³ in solvent, methiocarb at 23 mg/m³ in solvent, or methiocarb at 96 mg/m³ in solvent; exposure was for 6 h/day, five days per week for three weeks. The animals were examined daily and weighed weekly. Blood was taken from five animals per group at the end of the study and was used for haematological and clinical chemical tests; urinary analyses were also carried out. Plasma and erythrocyte cholinesterase activity was determined before treatment and after 5, 10, and 15 exposures for all groups except the air controls. At the end of the study, the animals were sacrificed and autopsied, selected organs were weighed and processed for histopathological examination, and brain acetylcholinesterase activity was measured.

No deaths were observed in any group. Animals at the highest concentration showed clinical signs of compound-related effects (tremor); no clinical signs were seen at lower concentrations. There body weight of males at the highest concentration was reduced by comparison with the air controls but not with the solvent controls. No change in haematological or clinical chemical parameters was seen that was attributable to the test material, with the exception of inhibition of cholinesterases. Plasma and brain cholinesterase activity was decreased at the highest concentration, brain acetylcholinesterase activity being 61 and 74 % of that in concurrent solvent controls in males and females, respectively. Some inhibition of plasma cholinesterase activity was seen at the intermediate concentration, and males at this concentration had an associated decrease in brain acetylcholinesterase activity (65% of concurrent control value). Erythrocyte acetylcholinesterase activity was less strongly affected than plasma enzyme, but marginal inhibition was observed in males at the highest concentration at week 1 (82% of concurrent solvent control value). There were no toxicologically significant alterations in organ weights, and no compound-related findings were noted on histopathological examination. The NOAEL was 6 mg/m³ on the basis of reduced brain acetylcholinesterase activity in males (Thyssen & Mohr, 1983).

Rabbits

Technical-grade methiocarb (purity, 99.2%) was applied to five male and five female chinchilla rabbits, daily for two weeks, at a dose of 500 mg; five males and five females served as controls.

The material was applied for 24 h/day, and new material was applied at the end of each 24-h period. After the two-week application period, the animals were observed for a further fortnight. The animals were inspected daily, and haematological and biochemical studies were carried out before treatment, at the end of treatment, and two weeks later. Clinical chemistry was restricted to liver function tests and urinary analysis; cholinesterase activity was not measured. No abnormal clinical signs were seen, and there was no effect on body-weight gain or any perturbation in haematological or clinical chemical variables (Kimmerle, 1969c).

Methiocarb (purity, 99.3%) was applied at doses of 0, 60, 150, or 375 mg/kg bw per day for 6 h/day to the skin of groups of five male and five female New Zealand white rabbits, and the site of application was occluded. The animals were examined twice daily, and any signs of skin irritation were scored. The rabbits were weighed twice weekly; food consumption was measured three times weekly until the last week, when it was measured four times. Blood was taken for haematological and clinical chemical analysis before treatment and pre-terminally. Plasma and erythrocyte cholinesterase activity was measured at the end of the 6-h exposure period on days 1, 7, 14, and 21; additionally, blood was taken 16 h after the end of exposure on these days from the group at the high dose. Animals were sacrificed on the day after the last treatment, at which time the brain was taken for measurement of acetylcholinesterase activity in a homogenate of the entire left half of the brain. Selected tissues were weighed, examined grossly, processed, and examined histopathologically.

Two animals at the low dose did not survive to the end of the study. No clinical signs related to the test material were seen, and it was not irritating. There was no differences between the groups in body weight, but food consumption appeared to be reduced in animals of each sex at the high dose, and particularly in males. Clinical chemical parameters did not differ between the groups, and, although one or two differences in haematological measurements were seen, none appeared to be related to treatment. Plasma cholinesterase activity appeared to be reduced in males at the high dose at 14 and 21 days. The measurements made 6 h after the end of exposure suggested that the inhibition was not reversed overnight. No intergroup differences in plasma cholinesterase activity were observed among females. Erythrocyte acetylcholinesterase activity was very variable but did not appear to be inhibited in a dose-related fashion. Intergroup differences were not observed in brain acetylcholinesterase activity. No gross or microscopic abnormality was observed that was attributable to the test material. The NOAEL was 150 mg/kg bw per day on the basis of reduced food consumption (Procter, 1988).

Methiocarb (purity, 97.5%) was applied to the skin of five male and five female New Zealand white rabbits at a single dose of 500 mg/kg bw per day for 6 h/day under occlusion. Five controls of each sex received saline. The animals were examined twice daily, and any signs of skin irritation were scored. They were weighed twice weekly, while food consumption was measured every two days. Blood was taken for haematological and clinical chemical tests before treatment and preterminally. Plasma and erythrocyte cholinesterase activity was measured at the end of the 6-h exposure period on days 1, 7, 14, and 21 and, in the test animals, 16 h later. Animals were sacrificed on the day after the last treatment, at which time the left half of the brain was taken for measurement of cholinesterase activity. Selected tissues were weighed, examined, processed, and examined histopathologically.

Two test animals removed their dressings and presumably ingested the material; these animals developed clinical signs of cholinergic poisoning, which disappeared within a few hours. No other compound-related clinical signs were seen, and no animal died before completion of the study. Treated females were lighter than controls throughout the study, and the food consumption of animals of each sex was reduced. A reduction in serum calcium and increased activities of alanine and aspartate aminotransferases were seen in females in comparison with concurrent controls. Plasma cholinesterase activity was lower than that before treatment in both males and females, but only inconsistently in comparison with concurrent controls. Erythrocyte acetylcholinesterase activity was reduced in males on day 1, at both 6 and 16 h, in comparison with pretreatment levels, but inconsistently in comparison with concurrent controls; it was concluded that compound-related inhibition of erythrocyte acetylcholinesterase activity had not occurred in either males or

females. Brain acetylcholinesterase activity was not inhibited. No abnormality was seen at autopsy or on histopathological examination. The design of this study was not appropriate for identifying a NOAEL (Procter, 1989).

Cats

Methiocarb at a dose of 5 mg/kg bw per day, given daily by gavage to cats, was reported to have no adverse effects (Kimmerle, 1960).

Dogs

Methiocarb was incorporated into the diet of groups of two male and two female beagles at a concentration of 0, 50, 100, or 250 ppm, equal to 0, 1.25, 2.5, or 6.25 mg/kg bw per day. The animals were examined daily and weighed fortnightly; plasma and erythrocyte cholinesterase activity was measured weekly. No clinical effects were seen, and body-weight gain was unaffected by treatment. There was no clear difference in plasma or erythrocyte cholinesterase activity between the test groups and concurrent controls. The small group size renders this study inappropriate for identifying a NOAEL (Root et al., 1963).

Chickens

Chickens (*Gallus gallus* Babcock 300) were fed diets containing a 9:1 mixture of methiocarb and methiocarb sulfoxide (based on studies of plant metabolism) at a dose of 20, 60, 120, or 360 ppm, equal to 2.5, 7.5, 15, and 45 mg/kg bw per day, over 28 days. Treatment decreased feed consumption in a dose-related fashion, and the body weights of birds at the two highest doses were decreased. Egg production was unaffected. Plasma cholinesterase activity was decreased by 40–50 % in comparison with concurrent controls at the three highest doses but was unaffected at the lowest dose (Strankowski & Minor, 1976).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female BOR:CFW1 mice received diets containing methiocarb (purity, 98.5%) at concentrations of 0, 67, 200, or 600 ppm, equal to 0, 15, 43, and 130 mg/kg bw per day in males and 0, 20, 57, and 170 mg/kg bw per day in females. Haematological and clinical chemical tests were performed on five animals of each sex per dose at 12 months and 10 animals of each sex per dose at 24 months, and on satellite groups of 15 mice of each sex per dose, which were sacrificed after one year and necropsied. Cholinesterase activity was determined in the plasma of five animals of each sex per dose at 1 and 12 months and on 10 animals of each sex per dose (or the survivors if fewer than 10) at the end of the study. Brain cholinesterase activity was determined at the end of the study. Animals that died or became moribund were autopsied, as were 10 mice of each sex per group at 12 months. All surviving animals were sacrificed at the end of the study and autopsied, and selected organs were weighed, examined, and processed for histopathological examination.

The appearance, behaviour, mortality rate, and food consumption of the animals were not affected at any dose, except for a slight decrease in body weight at the highest dose, throughout the study in males and up to week 30 in females. Overall survival in this study was not good: survival at 18 months was 74% of male controls, 47% males at the low dose, 59% males at the intermediate dose, 66% males at the high dose, 69% of female controls, 53% at the high dose, 64% at the intermediate dose, and 69% at the high dose. Intergroup differences in mortality rates did not appear to be compound-related, as mortality was higher in males receiving 67 or 200 ppm than in those given the high dose. Moreover, survival of females up to the end of the study was poorest for controls. All treated male mice had higher mean corpuscular haemoglobin concentrations than controls at 12 months, and males at the highest dose had higher mean corpuscular haemoglobin

values; similar changes were not seen in females. At 24 months, the mean corpuscular haemoglobin concentration of males at 200 and 600 ppm was decreased and the mean corpuscular haemoglobin value at 600 ppm, again, with no similar change in the females. Higher leukocyte counts were observed in all treated females at 24 months, but the authors ascribed this finding to high individual values; no perturbation of the differential count was seen.

No clinical chemical abnormalities were found at 12 months, but at 24 months the activity of alanine aminotransferase (ALAT) was increased in animals at 200 and 600 ppm. At one month, males at 200 and 600 ppm had reduced plasma cholinesterase activity (by 51 and 34% in comparison with concurrent controls), while reductions of 5% or less were seen at 12 months and 5–11% at 24 months. In females, plasma cholinesterase activity was inhibited by 24, 43, and 34% at the low, intermediate, and high doses, respectively, at one month; smaller reductions (< 12%) were observed at 12 months, and no cholinesterase inhibition was observed at 24 months. Erythrocyte acetylcholinesterase activity was not measured. Small reductions in brain acetylcholinesterase activity were observed, by 5, 11, and 10% in males at the low, intermediate, and high doses and by 3, 9, and 3% in females at the three doses, respectively. No compound-related change in organ weights was seen, nor was there any abnormality in gross of histological appearance that was related to treatment. Methiocarb was not tumorigenic. There was no NOAEL because of haematological changes at all doses in males at 12 months and in females at all doses at 24 months (Krötlinger & Janda, 1983; Krötlinger, 1989).

Rats

Methiocarb (purity, 98.9%) was admixed with the diet of groups of 60 Wistar TNO W.74 rats for two years at concentrations of 0, 67, 200, or 600 ppm, equal to 0, 3.3, 9.3, and 29 mg/kg bw per day for males and 0, 5, 14, and 42 mg/kg bw per day for females. The rats were inspected daily, and body weights were determined weekly for the first 26 weeks and then at fortnightly intervals. Haematological, clinical chemical, and urinary measurements were made in 10 animals of each sex per dose at 3, 6, 12, and 24 months. Cholinesterase activity in plasma and erythrocytes was determined one and two days after the start of the study and at 1, 2, 4, 8, 13, 26, 52, 78, and 105 weeks in 10 animals of each sex per dose. Brain acetylcholinesterase activity was determined at the end of the experiment on 10 animals of each sex per dose. Animals that died and those sacrificed *in extremis* were examined grossly and necropsied; when possible, tissues were taken for histopathological examination. The survivors at two years were also examined grossly and necropsied, and tissues were taken for weighing and histopathological examination.

None of the doses had any effect on the appearance, behaviour, or mortality rates of rats of either sex. The mortality rates at two years were 10–20% for males and 23–32% for females. There was no significant intergroup difference in food consumption. Weight gain was not depressed at 67 or 200 ppm in comparison with the controls; at 600 ppm, weight gain was slightly but consistently depressed throughout the period of administration of methiocarb, and at termination total body weight was reduced in the group at the high dose. At three months, an increased leukocyte count was seen in females at the highest dose and an increased reticulocyte count in females at the two higher doses. At six months, the mean corpuscular haemoglobin concentration was reduced in males at the highest dose, and some elevation in leukocyte count was found in males at 67 and 200 ppm. Red blood cell counts and haemoglobin and haematocrit values were decreased in females at the two higher doses, and reticulocytosis was observed in these groups. At 12 months, the mean corpuscular haemoglobin concentration was decreased in males at the intermediate dose and was increased in females at the highest dose; an increased reticulocyte count was seen in females at the high dose. At 24 months, no compound-related changes in haematological variables were seen.

Although a few intergroup differences were observed in biochemical tests, the only ones that appeared to be related to treatment were total protein concentration and cholinesterase activity. Total protein concentrations were raised in females at the two higher doses at six months and in males at the three highest doses at 12 months; at 24 months, the total protein concentration was similar in treated and control groups. ALAT activity in the blood was elevated in all three groups of treated females at 12 months and in females at the highest dose at two years, but at no time interval in males. Increased urea was found in plasma from males at the highest dose at three and

12 months and in females at 12 and 24 months. Plasma cholinesterase activity was depressed at the high dose at one day and from eight weeks onwards in males (except in the 52-week assay) and in females at one day and 1, 2, 4, and 13 weeks. The measurements of erythrocyte acetylcholinesterase activity were difficult to interpret: a statistically significant depression was found only at the low dose in males at 105 weeks and females at 78 weeks, and the degree of depression was small (5 and 6%, respectively). Males at the intermediate dose had significantly depressed activities at 8, 78, and 105 weeks, with 5, 7, and 8% depression, respectively. In females at this dose, significant depression was seen at four and 78 weeks (8 and 13% depression, respectively). In males at the highest dose, significant depressions were seen at 8, 13, 78, and 105 weeks (7, 7, 9, and 7%, respectively), and in females at this dose depression was seen at two days and 4 and 78 weeks (6, 8, and 11%, respectively). The Committee considered that none of these depressions was biologically significant. No depression of brain acetylcholinesterase activity was seen.

Males at the highest dose had decreased absolute weights of the thyroid, heart, lung, liver, spleen, and adrenals, but these were reflected only in reductions in the relative weights of the spleen and therefore probably reflect reduced body weight. In the females, only the absolute weight of the spleen was reduced, while the absolute weight of the thyroid was increased. As this finding was due to a single animal, it is unlikely to be attributable to the test material. No gross or histopathological abnormality related to treatment was seen at any dose. Methiocarb was not tumorigenic. The NOAEL was 67 ppm, equal to 3.3 mg/kg bw per day, on the basis of haematological changes at 3, 6, and 12 months (Krötlinger et al., 1981; Krötlinger, 1990).

Dogs

Groups of four beagle dogs and four bitches were fed methiocarb (purity, 98.4%) in the diet at 0, 5, 60, or 240 ppm; the group given 5 ppm group had been given 15 ppm for the first 15 days. These dietary concentrations were equivalent to daily doses of 0, 0.12, 1.5, and 6 mg/kg bw per day, ignoring the first 15 days of treatment of animals at the lowest dose. The animals were examined daily, and food consumption was recorded daily and body weight measured weekly. Clinical examinations including ophthalmoscopy were undertaken, and haematological and biochemical variables were measured in blood at weeks 0, 14, 27, 40, 53, 66, 79, 92, and 104; urine was also analysed. Erythrocyte and plasma cholinesterase activity was measured before the start of treatment and at weeks 2, 3, 4, 7, 10, 13, 27, 40, 53, 66, 79, 92, and 104, before feeding and 2 h afterwards. Acetylcholinesterase activity on the olfactory bulb of the brain was measured at sacrifice. Animals were examined grossly *post mortem*, and selected tissues were examined histologically.

One death occurred, of an animal at 5 ppm, which was considered not to be related to treatment. The only clinical findings were mild weakness of the hind limbs, trembling, reduced alertness, and some vomiting at the highest dose during the first 14 weeks of the study. The results of tests for reflexes and ophthalmic parameters were normal. Food intake was reduced in animals of each sex at the highest dose and in bitches at the intermediate dose, but the body weights were not significantly affected. Haematological and biochemical parameters were unaffected, apart from cholinesterase activity. Plasma cholinesterase activity was depressed at doses of 15 ppm and higher, and it was for this reason that this dose was reduced to 5 ppm. Depression of plasma cholinesterase activity was not seen at 5 ppm but occurred at the two higher doses. Erythrocyte and brain acetylcholinesterase activity was not consistently inhibited at any dose; the maximum inhibition of erythrocyte acetylcholinesterase activity was in animals at the high dose (17% for each sex) and at the intermediate dose (10% in dogs and 5% in bitches). Organ weights were unaffected, and no organ-specific toxicity observed. The NOAEL was 60 ppm, equivalent to 1.5 mg/kg bw per day, on the basis of clinical signs. The reduced food intake of bitches at the intermediate dose was not considered relevant (Hoffman & Schilde, 1980).

(d) *Genotoxicity*

The results of assays for the genotoxicity of methiocarb are shown in Table 2.

Table 2. Results of assays for the genotoxicity of methiocarb

End-point	Test object	Concentration	Purity (%)	Result	Reference
<i>In vitro</i> Reverse mutation ^a	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	4–2500 µg/plate	98.5	Negative	Herbold (1978)
Reverse mutation ^a	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	20–12 500 µg/plate	98.4	Negative	Herbold (1986)
DNA damage ^a	<i>E. coli pol A⁺</i> and <i>pol A⁻</i>	625–10000 µg/plate	98.6	Negative	Herbold (1983)
Gene mutation	Chinese hamster ovary cells, <i>hprt</i>	1.25–60 µg/ml	99.3	Negative	Lehn (1989)
Unscheduled DNA synthesis	Rat primary hepatocytes	0.1–100 µg/ml	98.8	Negative	Curren (1988)
Chromosomal aberration ^a	Chinese hamster ovary cells	4.92–497 µg/ml	99.4	Positive	Murli (1990)
Sister chromatid exchange ^a	Chinese hamster ovary cells	2–40 µg/ml	98.2	Negative	Putman (1986)
<i>In vivo</i> Micronucleus formation	Mice	5, 10, and 20 mg/kg bw twice orally	98.5	Negative	Herbold (1979a)
Dominant lethal mutation	Mice	6 mg/kg bw	98.5	Negative	Herbold (1979b)

^a With and without metabolic activation

(e) *Reproductive toxicity*

(i) *Multigeneration reproductive toxicity*

Rats

Groups of 10 male and 10 female FB 30 rats (Elberfield breed) received technical-grade methiocarb (purity, 99%) admixed with the diet at concentrations of 0, 30, 100, or 300 ppm, equivalent to 3, 10, and 30 mg/kg bw per day. The rats were weighed weekly. The F₀ generation was mated twice, first after 70 days of treatment and again after 149 days, to produce the F_{1a} litters, which were sacrificed, and the F_{1b} litters. Ten males and 10 females from each group of F_{1b} rats were used to produce the next generation and were again mated twice. The resultant F_{2a} rats were sacrificed, while 10 male and 10 female F_{2b} rats were mated twice to produce the F_{3a} and F_{3b} generations, which were sacrificed. The rats were weighed weekly, and the body weights of the offspring were measured at birth, five days after birth, one week after birth, and then weekly. The pups were examined grossly for malformations immediately after birth and during lactation. The F_{3a} young were killed four weeks after birth and the F_{3b} rats at three weeks. Blood samples were taken for analysis from the F₀ generation at the end of the preliminary treatment period and after rearing of the second litter (Löser, 1969).

In the F₀ generation, there was no significant difference in weight gain between the groups, and no treatment-related changes were seen in haematological parameters at either sampling time. Although ALAT and aspartate aminotransferase activities were higher in animals at the highest dose at the earlier sampling time, they were stated to be within the normal range for the laboratory; similar increases were not seen at the later sampling time. The gestation rate was lower at the highest dietary concentration, but was still within the normal range. The weight gain of F_{1a} pups

was similar in all groups, and no malformations were seen at sacrifice. After the second mating, there were no dose-related changes in litter size or pup weight and weight gain. No significant differences were seen between the groups of F_{1b} pups. Their weight gain after weaning was similar in all groups. After the first mating, the gestation rate was lower at the highest dietary concentration but was still within the normal range. The weight gain of F_{2a} pups was similar in all groups, and no malformations were seen at sacrifice. After the second mating, the mean weight of the group at 300 ppm was significantly lower than that of controls at the end of the four-week lactation period; however, there were no significant intergroup differences in body weight at sacrifice of the F_{2b} animals. After both matings of these rats, the gestation rate was similar and the litter size and pup weights were comparable in all groups. The body weights of the F_{3b} , but not the F_{3a} , groups at 100 and 300 ppm during lactation were lower than those of controls. This finding may be due to larger litter size. No abnormalities were found at birth or sacrifice in F_{3a} or F_{3b} pups, and no abnormalities were found at autopsy of the F_0 , F_{1b} , and F_{2b} generations. The NOAEL was 300 ppm, equivalent to 30 mg/kg bw per day, the highest dose tested (Löser (1970).

(ii) *Developmental toxicity*

Rats

Groups of 19–20 fertilized FB 30 rats received 10 oral doses of 0, 1, 3, or 10 mg/kg bw per day methiocarb (purity, 98.9%) by gavage on days 6–15 of gestation. The highest dose reduced weight gain, but no other effects were observed. No effects were seen on the number of implantations or resorptions or the weights of fetuses or placentas. No teratogenic effects were seen, nor was fetotoxicity observed. The NOAEL for maternal toxicity was 3 mg/kg bw per day, and that for fetal toxicity was 10 mg/kg bw per day, the highest dose tested (Lorke, 1971; see also Renhof, 1988).

Rabbits

Methiocarb (purity, 97.3%) was administered by gavage to groups of 17 pregnant New Zealand white rabbits at doses of 1, 3, or 10 mg/kg bw per day on days 6–18 of gestation. A group of 19 rabbits were used as controls. On day 29 of gestation, the rabbits were sacrificed and examined; the uterus was weighed, and the numbers of corpora lutea, implantation sites, and resorption sites, and the number and distribution of live and dead fetuses were noted. The weight and sex of the fetuses and placental weights were recorded and the fetuses examined for abnormalities. Rabbits receiving the highest dose showed clinical signs of cholinergic poisoning and initial marked weight loss. Weight gain was also reduced at 3 mg/kg bw per day towards the end of the study, but this was attributable to a single animal. Weight gain was unaffected at the lowest dose. The litter parameters were similar in all groups, and there was no indication of teratological effects. The NOAEL for maternal toxicity (clinical effects and weight loss at the highest dose) was 3 mg/kg bw per day. The NOAEL for fetotoxicity was 10 mg/kg bw per day, the highest dose tested (Tesh et al., 1981).

Methiocarb (purity, 99.4–99.6%) was applied under occlusion for 6 h/day to about 10% of the body area of groups of 16 mated chinchilla rabbits at doses of 0, 10, 50, or 250 mg/kg bw per day on days 6–18 post coitum. The animals were sacrificed after 28 days and the fetuses removed. One animal at the high dose was sacrificed because of a broken femur. No clinical signs were observed at any dose, nor was irritation of the skin noted. At the highest dose, reduced food consumption was observed between 11 and 15 days *post coitum* and body-weight loss between 6 and 8 days *post coitum*, whereas at the lower doses, no such effect was seen. The numbers of corpora lutea, implantations, pre- and post-implantation losses, and live fetuses were not affected by treatment. There was a slight decrease in mean fetal weight at the highest dose (4%), and slight retardation of skeletal development was noted at this dose, as shown by incomplete or no ossification of the phalangeal nuclei. No effects on the fetuses were seen at lower doses. The NOAEL for both maternal and fetal toxicity was 50 mg/kg bw per day (Dotti & Biedermann, 1992).

(f) *Special studies*(i) *Dermal and ocular irritation and dermal sensitization*(ii) *Neurotoxicity*

In a poorly described study, no persistent paralytic effects were produced in hens (*Gallus gallus*) of unstated strain given a single dose of methiocarb by gavage and observed for 14 days (DuBois, 1962).

Methiocarb (purity, 98.5%) was administered twice at a dose of 380 mg/kg bw (approximately the LD₅₀) to 20 white Leghorn laying hens, aged 15–20 months, after an intramuscular injection of 50 mg/kg bw atropine sulfate. After an interval of 21 days, the 18 surviving hens were treated again, with the same doses of atropine and methiocarb. The 16 survivors of the second treatment were sacrificed three weeks later, and brain, spinal cord, and portions of peripheral nerve were excised and processed for histological examination. Five positive controls were given tri-*ortho*-cresyl phosphate as a single oral dose and sacrificed three weeks later. The hens treated with methiocarb exhibited brief cholinergic signs and were lethargic, but none showed ataxia or paralysis. At examination, *post mortem* there were no histopathological changes indicative of polyneuropathy in either the central or peripheral nervous system, whereas the hens treated with tri-*ortho*-cresyl phosphate showed the classical signs of delayed polyneuropathy and mild but characteristic signs of delayed polyneuropathy on histopathological examination (Thyssen & Schilde, 1978).

(iii) *Antidotes*

A study was carried out in which the effect of antidotes to an oral LD₅₀ of methiocarb was investigated in rats. The regimens used were atropine at 50 mg/kg bw, pralidoxime (salt unstated) at 50 mg/kg bw, obidoxime at 20 mg/kg bw, atropine and pralidoxime at 50 mg/kg bw each, or atropine at 50 mg/kg bw with obidoxime at 20 mg/kg bw. All the antidotes were given intraperitoneally just before clinical signs became evident, i.e. 1–2 min after administration of methiocarb. The LD₅₀ values were 67 mg/kg bw without antidotes, 470 mg/kg bw with atropine, 190 mg/kg bw with pralidoxime, 220 mg/kg bw with obidoxime, 500 mg/kg bw with pralidoxime and atropine, and 510 mg/kg bw with obidoxime and atropine. Thus, pralidoxime and obidoxime added little to the antidotal effect of atropine (Kimmerle, 1966a).

In mice, 10 mg/kg bw of atropine increased the LD₅₀ of methiocarb by sevenfold, while pralidoxime methiodide at 100 mg/kg bw increased the LD₅₀ 1.3 times. Administration of the two antidotes together increased the LD₅₀ by 21 times. Obidoxime at 50 mg/kg bw increased the LD₅₀ 3.1 times, while obidoxime plus atropine increased it by 9.1 times (Kohgo, 1970).

Atropine sulfate at 50 mg/kg bw administered within 10 min of methiocarb intraperitoneally increased the LD₅₀ of methiocarb given orally to rats by more than sixfold, and tetraethyl ammonium chloride given by the same route increased it by approximately fourfold; however, the combined effect of the two antidotes was less than that of atropine alone (Kimmerle, 1971).

In the study of Thyssen and Schilde (1978) in hens at approximately the LD₅₀ (see above), atropine appeared to have a powerful antidotal effect.

(iv) *Interaction with other pesticides*

When an intraperitoneal dose equal to 50% of the LD₅₀ of methiocarb was given to female Sprague-Dawley rats in combination with another pesticide at a dose equal to 50% of the LD₅₀, the combined mortality was less than 50% in all cases. The highest combined mortality rates (> 40 to < 50%) were seen with EPN, Guthion, mevinphos, and carbaryl. Combined mortality rates of > 20 to < 40% were found with parathion, parathion-methyl, disulfoton, malathion, carbophenothion,

tributyl phosphorotrithioite, and ethion. The mortality rates seen with the remaining insecticides (demeton, dioxathion, and schradan) were 5–20% (DuBois & Raymund, 1961b). In another study of similar design, the combined mortality rates were 55% with trichlorfon, 50% with propoxur, 35% with coumaphos, 30% with oxydemeton-methyl, and 20% with fenthion (DuBois & Raymund, 1961c).

3. Observations in humans

The 250 workers in two plants manufacturing methiocarb over about 20 years were subjected to annual medical examinations and assays of cholinesterase activity in whole blood. No adverse health effects or changes in laboratory parameters were observed (Faul, 1993).

Comments

Methiocarb appeared to have been well absorbed in a small study of absorption, distribution, metabolism, and excretion of the radiolabelled compound in rats. More than 70% of the administered radiolabel was excreted within 48 h, mostly in the urine. Methiocarb was extensively metabolized. The main route of metabolism in both animals and plants appears to be to methiocarb phenol, methiocarb phenol sulfoxide, and methiocarb phenol sulfone. In some studies, methiocarb sulfoxide was also found.

In single-dose and short-term studies in rats, methiocarb administered by gavage inhibited plasma and erythrocyte cholinesterase activity. In the short-term study, plasma, erythrocyte, and brain cholinesterase activities were depressed at 10 mg/kg bw per day; the NOAEL for this effect was 3 mg/kg bw per day. In a short-term study of the ability of methiocarb or its sulfoxide to inhibit cholinesterase activity in rats, the NOAEL for inhibition of erythrocyte acetylcholinesterase activity was 0.5 mg/kg bw per day for methiocarb, but a NOAEL was not identified for the sulfoxide. In a 29-day study in which dogs were given methiocarb or methiocarb sulfoxide in gelatine capsules at 0.05 or 0.5 mg/kg bw per day, both plasma and erythrocyte cholinesterase activities were inhibited by both treatments.

The acute toxicity of methiocarb given by a number of routes has been measured in a number of species. The oral LD₅₀ in fasting rats ranged from 13 to 130 mg/kg bw. The oral LD₅₀ values for methiocarb phenol, methiocarb phenol sulfoxide, and methiocarb phenol sulfone in rats are all greater than 1 g/kg bw, as is that of methiocarb sulfone, but that of methiocarb sulfoxide is between 6 and 43 mg/kg bw.

WHO has classified methiocarb as moderately hazardous (WHO, 1996).

The short-term toxicity of methiocarb has been tested in rats, rabbits, cats, dogs, and chickens. Few of these studies were appropriate for identifying NOAEL values and, of those that were, a study in rats was carried out by inhalation and that in rabbits by dermal application. In the study in rats, which were exposed by inhalation, for five days per week for three weeks, no findings related to treatment were seen on histopathological examination but depressed brain acetylcholinesterase activity was observed at the highest dose in animals of each sex and in males at the intermediate dose. Consequently, the NOAEL was 6 mg/m³ per day. In a 21-day study of the dermal toxicity of methiocarb in rabbits, the substance was applied for 6 h/day at 0, 60, 150, or 375 mg/kg bw per day. Plasma cholinesterase activity was reduced in males at the highest dose, but no significant differences were seen between groups in the activities of erythrocyte and brain acetylcholinesterase. The NOAEL was 150 mg/kg bw per day on the basis of reduced food consumption at the highest dose.

In a long-term study of toxicity in mice, methiocarb was administered at dietary concentrations of 0, 67, 200, or 600 ppm. A NOAEL was not identified because haematological changes were observed in all treated males at 12 months and in all treated females at 24 months. The LOAEL was 67 ppm, equal to 15 mg/kg bw per day, on the basis of minor haematological changes. Methiocarb was not carcinogenic in mice.

In a two-year study of toxicity, rats received methiocarb at dietary concentrations of 0, 67, 200, or 600 ppm. The NOAEL was 67 ppm, equal to 3.3 mg/kg bw per day, on the basis of

haematological changes at 3, 6, and 12 months. Methiocarb was not carcinogenic.

In a two-year study of toxicity, dogs were fed methiocarb in the diet at 0, 5, 60, or 240 ppm. The NOAEL was 60 ppm, equivalent to 1.5 mg/kg bw per day, on the basis of reversible clinical signs at the next highest dose, which were not observed after 15 weeks.

Three studies of developmental toxicity were available, one in rats and two in rabbits. Fertilized rats received methiocarb by gavage on days 6–15 of gestation at 0, 1, 3, or 10 mg/kg bw per day. The NOAEL for maternal toxicity was 3 mg/kg bw per day on the basis of reduced body-weight gain at the highest dose. As no fetotoxicity was observed, the NOAEL for this end-point was 10 mg/kg bw per day, the highest dose tested. In a study of developmental toxicity in rabbits, pregnant animals received methiocarb at 0, 1, 3, or 10 mg/kg bw per day by gavage on days 6–18 of gestation. The NOAEL was 3 mg/kg bw per day for maternal toxicity on the basis of clinical effects and weight loss at the highest dose. As no fetotoxicity or teratogenicity was observed, the NOAEL for these end-points was 10 mg/kg bw per day, the highest dose tested. In a further study, rabbits received methiocarb by dermal application at 0, 10, 50, or 250 mg/kg bw per day for 6 h/day on days 6–18 of gestation. The NOAEL for both maternal and fetal toxicity was 50 mg/kg bw per day on the basis of reduced maternal food consumption and some decrease in mean fetal weight at the highest dose; slight retardation of fetal development was also observed. A multigeneration study of reproductive toxicity was undertaken in rats given methiocarb at dietary concentrations of 0, 30, 100, or 300 ppm. The NOAEL was 300 ppm, equivalent to 30 mg/kg bw per day, as no effect clearly related to treatment was observed. No teratogenic effect was observed in any of these studies.

Methiocarb has been tested for genotoxicity in an adequate battery of tests *in vitro* and *in vivo*. The Meeting concluded that methiocarb is not genotoxic.

Methiocarb did not cause skin sensitization in studies conducted by either the Magnusson and Kligman or the Beuhler technique.

In an early study of neurotoxicity in hens, methiocarb did not cause delayed polyneuropathy of the organophosphorus type. Atropine has consistently been shown to be an effective antidote for methiocarb, while the effects of pyridinium oximes were somewhat inconsistent.

The Meeting established an ADI of 0–0.02 mg/kg bw on the basis of the NOAEL of 1.5 mg/kg bw per day in the two-year study of toxicity in dogs and a safety factor of 100. This ADI results in a further safety factor of 10 on the LOAEL in the long-term study of toxicity in mice.

An acute RfD was allocated on the basis of the NOAEL of 1.5 mg/kg bw per day in the two-year study in dogs, because the signs observed were acute, and a safety factor of 100. Of the shorter studies in dogs, neither the 29-day nor the 12-week study was considered by the Meeting to be adequate for the purpose of establishing a NOAEL.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	No NOAEL; LOAEL: 67 ppm, equal to 15 mg/kg bw per day (long-term study of toxicity)
Rat:	67 ppm, equal to 3.3 mg/kg bw per day (long-term study of toxicity) 300 ppm, equivalent to 30 mg/kg bw per day (maternal and fetal toxicity in a study of reproductive toxicity) 3 mg/kg bw per day (maternal toxicity in a study of developmental toxicity) 10 mg/kg bw per day (developmental toxicity)
Rabbit:	3 mg/kg bw per day (maternal toxicity in a study of developmental toxicity) 10 mg/kg bw per day (developmental toxicity)
Dog:	60 ppm, equivalent to 1.5 mg/kg bw per day (two-year study of toxicity)

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.02 mg/kg bw

Studies that would be useful for continued evaluation of the compound

1. A modern study of absorption, distribution, metabolism, and excretion
2. A modern multigeneration study of reproductive toxicity
3. Further observations in humans

List of end points relevant for setting guidance values for dietary and non-dietary exposure

<i>Absorption, distribution, excretion, and metabolism in mammals</i>			
Rate and extent of oral absorption		No data	
Dermal absorption		No data	
Distribution		No data	
Potential for accumulation		Not likely to accumulate	
Rate and extent of excretion		73 to > 90% within 48 h	
Metabolism in animals		Sulfoxidation and loss of carbamate side-chain	
Toxicologically significant compounds (animals, plants and environment)		Parent compound and methiocarb sulfoxide	
<i>Acute toxicity</i>			
Rat: LD ₅₀ , oral		13–135 mg/kg bw	
Rat: LD ₅₀ , dermal		350 to > 5000 mg/kg bw	
Rat: LC ₅₀ inhalation		> 300 mg/m ³	
Skin irritation		Not irritating	
Eye irritation		Not irritating	
Skin sensitization		Not sensitizing	
<i>Short-term toxicity</i>			
Target/critical effect		Clinical signs	
Lowest relevant oral NOAEL		Dog: 1.5 mg/kg bw per day	
Lowest relevant dermal NOAEL		Rabbit: 150 mg/kg bw per day	
Lowest relevant inhalation NOAEL		Rat: 6 mg/m ³	
<i>Genotoxicity</i>		Not genotoxic	
<i>Long-term toxicity and carcinogenicity</i>			
Target/critical effect		Clinical signs	
Lowest relevant NOAEL		Dog: 1.5 mg/kg bw per day	
Carcinogenicity		Not carcinogenic	
<i>Reproductive toxicity</i>			
Reproductive target/critical effect		No effect	
Lowest relevant reproductive NOAEL		Rat: 30 mg/kg bw per day	
Developmental target/critical effect		Maternal toxicity: reduced weight gain; no fetotoxicity observed	
Lowest relevant developmental NOAEL		Rabbit: 3 mg/kg bw per day	
<i>Neurotoxicity/Delayed neurotoxicity</i>		Does not cause delayed polyneuropathy	
<i>Other toxicological studies</i>		No data	
<i>Medical data</i>		No data	
Summary	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Dog, 2 years	100
Acute reference dose	0.02 mg/kg bw	Dog, 2 years	100

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