

**TOXICOLOGICAL MONOGRAPHS  
AND MONOGRAPH ADDENDA**

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## CARBOSULFAN

*First draft prepared by  
G. Wolterink and P.H. van Hoeven Arentzen  
Centre For Substances and Integrated Risk Assessment  
National Institute of Public Health and the Environment, Bilthoven, The Netherlands*

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### Explanation

Carbosulfan is a carbamate insecticide that acts by inhibiting the activity of acetylcholinesterase. It was evaluated by the Joint Meeting in 1984 and 1986. A toxicological monograph was prepared by the Joint Meeting in 1984 and a monograph addendum was prepared in 1986 (Annex 1, references 43, 47). In 1986, an acceptable daily intake (ADI) of 0–0.01 mg/kg bw was established on the basis of a NOAEL of 1.3 mg/kg bw per day in a 2-year study in mice, a NOAEL of 1.0 mg/kg bw per day in a 2-year study in rats, and a NOAEL of 1.3 mg/kg bw per day in a 6-month study in dogs. One of the metabolites of carbosulfan is carbofuran, which is itself used as a pesticide and which was evaluated by JMPR in 1976, 1979, 1980, 1982, 1996 and 2002. The 1996 JMPR established an ADI of 0–0.002 mg/kg bw and the 2002 JMPR established an acute reference dose (RfD) of 0.009 mg/kg bw for carbofuran.

Carbosulfan was re-evaluated by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting reviewed new data on carbosulfan that had not been considered previously and relevant data from the

previous evaluation. Conclusions of studies evaluated by the JMPR in 1984 and that were not available for the present evaluation are included.

### Evaluation for acceptable daily intake

#### 1. Biochemical aspects

##### 1.1 Absorption, distribution and excretion

###### *Rats*

The kinetics of [<sup>14</sup>C]carbosulfan (purity, >97%) were studied in male and female Sprague-Dawley rats, in a study that complied with the United States Environmental Protection Agency (EPA) guideline 85-1. Carbosulfan was labelled either in the phenyl or in the dibutylamino moiety. In a preliminary test it was shown that excretion of radiolabel via expiration only occurred when carbosulfan was labelled in the dibutylamino group. The definitive experimental design was based on the preliminary test. Groups of five male and five female rats received unlabelled carbosulfan as a single oral dose at 4 or 30 mg/kg bw, or as repeated oral doses (4 mg/kg bw per day for 14 days), followed by [<sup>14</sup>C]carbosulfan as a single oral dose at 4 mg/kg bw on day 15. Corn oil was used as vehicle. Urine, cage rinses and faeces were collected at 0–4, 4–8, 8–12, 12–24, 24–36 h intervals and every 24 h thereafter for 168 h. At 168 h after dosing, the rats were killed, blood was sampled and bone (femur), brain, fat, kidneys, liver, lungs, muscle (thighs), ovaries, testes, heart, spleen, skin (shaven, dorsal), uterus and remaining carcass were collected. For rats treated with carbosulfan labelled in the dibutylamino group, expired carbon dioxide was collected. Statements of compliance with good laboratory practice (GLP) and quality assurance (QA) were provided.

Total recovery of radioactivity was 90–98%. No marked sex differences in patterns of excretion were observed. The major route of excretion was in the urine, excretion also occurring in the faeces in expired air. Patterns of excretion were similar in rats treated with carbosulfan at a single low or high dose. In the rats treated with single low or high doses of phenyl-labelled carbosulfan, 72–83% of the radiolabel was excreted in the urine. In the groups given dibutylamine-labelled carbosulfan at a low or high dose, 65–66% and 10–17% of the radiolabel was excreted in the urine and expired air, respectively. Total excretion of radiolabel in the faeces of rats treated with single low or high doses was about 8–22%. Excretion was relatively rapid. Most (80–90%) radiolabel was excreted within 48 h in animals at the lower dose and 72 h in animals at the higher dose. Excretion of radiolabel in the urine tended to be higher (phenyl group: 79–88%, dibutylamino group 71%) in rats receiving repeated doses of carbosulfan at 4 mg/kg bw than in rats treated with a single (low) dose at 4 mg/kg bw, while excretion in faeces tended to be lower (5–15%) in rats receiving repeated doses of carbosulfan at 4 mg/kg bw, indicating that absorption was slightly increased. Furthermore, in the rats treated with repeated doses, the rate of excretion of radiolabel was increased, i.e. 80–87% of the radiolabel was excreted within 24 h in the rats treated with repeated doses versus 72–78% in the those treated with a single low dose, which indicates that induction of metabolism may have occurred. At 168 h, <0.3% of the administered dose remained in blood and tissue, and up to about 2% remained in the carcass. During the first 24 h after dosing, rats at the highest dose showed behavioural signs indicative of cholinesterase inhibition. Remarkably, a high proportion (7–11%) of administered radiolabel was found in the cage wash of animals treated with carbosulfan labelled on the phenyl group, while in animals treated with carbosulfan labelled

on the dibutylamino group only 1–3% of the radiolabel was found in the cage wash (Fang & El Naggar, 1995).

### *Goats*

The kinetics of [<sup>14</sup>C]carbosulfan was studied in lactating Nubian goats (aged 2 years; body weight, 36–43 kg), in a study that complied with EPA guideline 171-4(a)(3). Carbosulfan was labelled with <sup>14</sup>C either in the phenyl ring (purity, 99.2%) or in the dibutylamino side-chain (purity, 98.8%). Gelatin capsules containing carbosulfan at a dose of 44.7 mg/day (phenyl label) or 40.9 mg/day (dibutylamino label) (approximate dietary concentration, 25 mg/kg) were administered orally by balling gun to groups of two goats, once daily for 7 days. A single goat received only vehicle (cellulose). Urine and faeces were collected daily and milk was collected twice daily. Blood was collected on days –1, 1, 3, and 7, immediately before dosing and immediately before sacrifice. The animals were killed 22 h after the last dose and edible tissues (liver, kidney, leg and lumbar muscle, peripheral and omental fat) were collected and total radioactive residue was determined. Excretion of radiolabel in expired air was not measured. Statements of compliance with GLP and QA were provided.

The major route of elimination was in the urine. For the goats receiving carbosulfan labelled on the phenyl group, excretion of radiolabel was 81–84% in urine, 7% in faeces, 1% in cage rinse and 0.2% in milk. For the goats receiving carbosulfan labelled on the dibutylamino side-chain, the pattern of excretion was 66–70% in urine, 3–4% in faeces, 0.3–0.4% in cage rinse and 2–3% in milk. For carbosulfan labelled on the phenyl group, total radioactive residue in tissue was highest in kidney (0.15–0.21 mg/kg) and liver (0.06 mg/kg). For carbosulfan labelled on the dibutylamino side-chain, total radioactive residue was highest in omental fat (1.1–1.3 mg/kg), liver (1.0–1.3 mg/kg), kidney (0.7–0.8 mg/kg) and peripheral fat (0.7–0.8 mg/kg). In animals of both groups, concentrations of radiolabel in the blood increased over 7 days, and highest concentrations of radiolabel in blood (phenyl group, 0.02–0.04 mg/kg; dibutylamino side-chain, 0.24–0.27 mg/kg) were observed just before termination (Curry & Weintraub, 1996).

### *(a) Biotransformation*

#### *Rats*

The metabolism of [<sup>14</sup>C]carbosulfan (purity, >97%) was studied in male and female Sprague-Dawley rats in vivo, in a study that complied with EPA guideline 85-1. Carbosulfan was labelled either in the phenyl moiety or in the dibutylamino moiety. Groups of five male and five female rats received unlabelled carbosulfan as a single oral dose at 4 (low) or 30 (high) mg/kg bw, or as repeated oral doses at 4 mg/kg bw per day for 14 days, followed by [<sup>14</sup>C]carbosulfan as a single oral dose at 4 mg/kg bw by gavage on day 15. Control rats received vehicle only (corn oil). Urine, cage rinses and faeces were collected at 0–4, 4–8, 8–12, 12–24, 24–36 h intervals and every 24 h thereafter for 168 h. At 168 h after dosing, the rats were killed, blood was sampled and bone (femur), brain, fat, kidneys, liver, lungs, muscle (thighs), ovaries, testes, heart, spleen, skin (shaven, dorsal), uterus and remaining carcass were collected. For rats treated with carbosulfan labelled in the dibutylamino side-chain, expired CO<sub>2</sub> was collected. Urine, faeces and exhaled air were analysed for radiolabel by liquid scintillation counting (LSC). Metabolites were identified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Identity of the major metabolites was confirmed by gas chromatography-mass spectrometry (GC-MS). Statements of compliance with GLP and QA were provided.

**Table 1. Quantitative excretion of carbosulfan and metabolites (% of administered radioactivity)**

	Dose (mg/kg bw)											
	4 (single dose)				4 (repeated doses)				30 (single dose)			
	Urine		Faeces		Urine		Faeces		Urine		Faeces	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
<i><sup>14</sup>C-phenyl label</i>												
Carbosulfan	ND	ND	2.5	3.4	ND	ND	0.9	0.4	ND	0.3	1.7	4.4
3-OH-7-phenol	14.2	12.7	1.2	0.7	25.3	25.6	0.9	0.3	17.9	17.9	0.6	0.5
3-OH-carbofuran	11.0	10.0	6.4	3.8	17.6	20.1	4.5	1.0	16.0	11.5	1.3	1.9
3-keto-7-phenol	20.3	26.3	0.4	0.3	14.4	20.7	0.2	0.1	25.7	23.9	0.2	0.4
7-phenol	23.5	22.8	0.4	0.3	8.8	11.6	0.1	0.0	7.3	4.8	0.1	0.3
<i><sup>14</sup>C-dibutylamino label</i>												
Carbosulfan	ND	ND	6.3	8.3	ND	ND	3.5	4.2	ND	ND	4.1	6.9
Dibutylamine	33.9	36.2	4.5	6.3	37.5	42.2	3.4	2.5	36.3	46.5	2.7	4.3
OH-dibutylamine	23.8	22.5	ND	ND	25.8	24.7	ND	ND	23.5	17.1	ND	ND

From Fang & El Naggar (1995)

ND, not detected

In total, 10 metabolites were identified. The distribution of radiolabel among the major metabolites is presented in Table 1. Other metabolites identified were carbofuran, 5-OH-carbofuran, 3-keto-carbosulfan, 3-OH-carbosulfan, 3-keto-carbosulfan-sulfone and 3-keto-carbofuran. Each of these metabolites or the seven unidentified metabolites was present at <5% of the total administered dose. The authors postulated that metabolites of the dibutylamino moiety may be incorporated in fatty acids. The metabolites resulting from hydrolysis and oxidation were excreted mainly as sulfate/glucuronide conjugates in urine. There was no obvious sex difference in metabolite formation. In the rats treated with single low or high doses of carbosulfan, the relative quantities of metabolites were similar. Compared with rats treated with single doses, the rats treated repeatedly with a low dose of carbosulfan had more 3-OH-7 phenol and 3-OH-carbofuran, and less 3-keto-7-phenol and 7-phenol. This may indicate that repeated dosing leads to induction of metabolizing enzyme systems. A proposed metabolic pathway is depicted in Figure 1 (Fang & El Naggar, 1995).

In a study reported in the public literature, the biotransformation of carbosulfan in the female rat stomach was assessed at short intervals after oral administration. Three rats received <sup>14</sup>C-labelled carbosulfan, dissolved in 0.2 ml of propylene glycol, at a dose of 30 mg/kg bw. The rats were killed 15, 35 or 80 min after treatment, the stomach was removed and the stomach contents were analysed by TLC. Total recovery of radioactivity was 75.7%, 55.1% and 44.6% at 15, 35 and 80 min, respectively. Of the recovered radioactivity, 60%, 47% and 50% represented carbosulfan at 15, 35 and 80 min respectively. Major biotransformation products at 15, 35 and 80 min, respectively, were carbofuran (15%, 20% and 16%), biscarbofuran *N,N'*-disulfide (11%, 17% and 18%) and an unidentified compound (7%, 8% and 9%); the data indicate that carbosulfan is relatively stable in the stomach (Umetsu & Fukuto, 1982).

### Goats

The kinetics of [<sup>14</sup>C]carbosulfan were studied in lactating Nubian goats (aged 2 years; body weight, 36–43 kg), in a study that complied with EPA guideline 171-4(a)(3). Carbosulfan was labelled with <sup>14</sup>C, either in the phenyl ring (purity, 99.2%) or in the dibutylamino



**Table 2. Quantitative distribution of metabolites of carbosulfan in goats (% of total radioactive residue in the sample)**

Metabolite	Sample				
	Milk	Liver	Kidney	Fat	Muscle
<i><sup>14</sup>C-phenyl label</i>					
3-OH-carbofuran	34.2	9.5	21.5	ND	ND
3-OH-7-phenol	21.1	15.6	13.3	ND	ND
3-keto-7-phenol	29.9	3.0	9.3	ND	ND
7-phenol	9.2	4.6	8.9	ND	ND
Minor metabolites	1.2	4.4	7.6	ND	ND
Characterized organosolubles	2.3	17.3	17.4	ND	ND
Protein-associated metabolites	NA	22.6	2.5	ND	ND
Polar aqueous metabolites	0.7	10.4	18.4	ND	ND
Non-extractable residues	1.4	12.7	2.1	ND	ND
<i><sup>14</sup>C-dibutylamino label</i>					
Aminobutanols	29.7	8.1	11.9	0.8	ND
Dibutylamine and related metabolites	6.7	13.4	10.5	0.6	9.6
Natural constituents	30.2	29.1	13.8	87.3	32.0
Non-conjugated amines	11.8	6.3	24.3	ND	5.9
Conjugated or bound amines	10.5	18.0	12.3	ND	14.7
Lipophilic metabolites	0.6	1.3	4.5	0.5	1.2
Polar aqueous metabolites	7.6	16.6	18.5	0.2	26.5
Non-extractable residues	2.9	7.2	4.2	10.5	10.0

From Curry & Weintraub (1996)

NA, not applicable; ND, not detected

## 2. Toxicological studies

### 2.1 Acute toxicity

#### (a) Oral, dermal and inhalation administration

For the present evaluation, no studies of acute toxicity with technical-grade carbosulfan were available. All available studies of oral, inhalatory and dermal toxicity were performed with formulations of carbosulfan that contained 25–50% active ingredient (a.i.). Data on the composition of the formulations were not provided in the studies. The acute toxicity of formulations containing carbosulfan is summarized in Table 3. The LD<sub>50</sub>s for the formulations and the recalculated values for the active ingredient are presented, assuming that the active ingredient is responsible for the toxic effect. In most of the studies with carbosulfan administered orally, the LD<sub>50</sub> tended to be lower for females than for males. In general, the clinical signs observed resemble those of cholinesterase inhibition. Studies of acute toxicity with technical-grade carbosulfan were evaluated by the JMPR in 1984. The oral LD<sub>50</sub>s in rats ranged from 90 to 250 mg/kgbw. The LD<sub>50</sub> for carbosulfan was >2000 mg/kgbw in rabbits treated dermally, and the LC<sub>50</sub> was 0.61 mg/l for carbosulfan in rats treated by inhalation.

The Meeting noted that in the studies of dermal toxicity the formulation was moistened with water or saline. In view of the high lipophilicity of carbosulfan ( $\log p_{ow} = 5.4$ ), it is not certain that good contact with the skin was maintained with the vehicles used.

#### (b) Ocular irritation

For the present evaluation, no studies of ocular irritation with pure or technical-grade carbosulfan were available. Five studies of ocular irritation with formulations of carbosul-

**Table 3. Acute toxicity of formulations containing carbosulfan**

Species	Strain	Sex	Formulation code <sup>a</sup>	Purity (%)	Route and vehicle	LD50 (mg/kg bw) or LC50 (mg/l)		Reference
						Formulation	Active ingredient	
Mouse	SW	Male	25 ST	28.9	Oral, in water	1077	311	Freeman (1985b) <sup>b</sup>
		Female				869	251	
Rat	SD	Male	4 EC	49.7	Oral, undiluted	69	34	Seaman (1981) <sup>d</sup>
		Female				≤55	≤27	
Rat	COBS(SD)	Male & Female	40 DB	40	Oral, in corn oil	>50	>20	Sabol (1981a) <sup>d</sup>
Rat	SD	Male	40 DB	40	Oral, in corn oil	348	134	Freeman (1984a) <sup>b</sup>
		Female				159	64	
Rat	SD	Male	25 ST	28.9	Oral, in water	278	80	Freeman (1985a) <sup>b</sup>
		Female				107	31	
Rat	Wistar	Male	35 STD	35	Oral, in water	113	40	Daamen (1991a) <sup>b</sup>
		Female				147	51	
Rat	SD	Male	25 STW	25	Oral, in water	258	65	Freeman (1989a) <sup>b</sup>
		Female				120	30	
Rat	Him:OFA	Male & Female	25 CS	25	Oral, in water	238	60	Klein (1993a) <sup>b</sup>
Rat	Wistar	Male & Female	400 SC	40.3	Oral, in water	42	17	Mello dos Santos (1998a) <sup>c</sup>
Rat	Wistar	Male & Female	35 STD	35	Dermal, in water	>2000	>700	Daamen (1991b) <sup>b</sup>
Rat	Him:OFA	Male & Female	25 CS	25	Dermal, undiluted	>2000	>700	Klein (1993b) <sup>b</sup>
Rat	Wistar	Male	400 SC	40.3	Dermal, in water	563	227	Mello dos Santos (1998b) <sup>c</sup>
		Female				688	269	
Rabbit	NZW	Male & Female	40 BD	40	Dermal, in saline	>200	>80	Sabol (1981b) <sup>d</sup>
Rabbit	NZW	Male & Female	25 ST	28.9	Dermal, in saline	>2000	>578	Freeman (1985c) <sup>b</sup>
Rabbit	NZW	Male & Female	25 STW	25	Dermal, in saline	>2000	>500	Freeman (1989b) <sup>b</sup>
Rat	CrI:CD	Male & Female	40 DB	40	Inhalation (1 h) <sup>h</sup>	<5.0	<2	Morgan (1981) <sup>d</sup>
Rat	CrI:CD	Male & Female	4 EC	47.2	Inhalation (1 h) <sup>d</sup>	<2.2	<1	Horath (1982) <sup>b</sup>
Rat	SD	Male & Female	25 STW	25	Inhalation (4 h) <sup>a</sup>	>0.11	>0.028	Blagden (1997) <sup>b</sup>
Rat	SD	Male	25 ST	28.9	Inhalation (4 h) <sup>f</sup>	0.26	0.075	Dudek (1985)
		Female				0.10	0.029	
Rat	SD	Male & Female	25 STD	35	Inhalation (1 h) <sup>i</sup>	>0.43	>0.15	Signorin (1993) <sup>b</sup>
Rat	Wistar	Male	400 SC	40.3	Inhalation (4 h) <sup>g</sup>	1.37	0.55	Mello dos Santos (1998c) <sup>c</sup>
		Female				1.72	0.69	

NSW, New Zealand white; SD, Sprague-Dawley

<sup>a</sup>All formulations are named "Marshal", followed by the codes indicated in the table

<sup>b</sup>Statements of compliance with GLP and QA were provided

<sup>c</sup>A statement of compliance with GLP was provided

<sup>d</sup>A statement of compliance with QA was provided

<sup>e</sup>Nose only; mass median aerodynamic diameter (MMAD), 7.2 μm; inhalable fraction, 16.6%

<sup>f</sup>Whole body; MMAD not presented

<sup>g</sup>Nose only; actual concentration and MMAD aerosol not measured

<sup>h</sup>Whole body; actual concentration and MMAD not assessed

<sup>i</sup>Whole body; gravimetric concentration, 0.43 mg/l; MMAD, 8.74 μm

fan were performed. The proportion of active ingredient in these formulations ranged from 25 to 40.3%.

In the studies of ocular irritation in rabbits, performed in compliance with guideline 81-4 and OECD 405, the irritating properties of Marshal 25 STW (25% a.i), Marshal 25 ST (28.9% a.i.), Marshal 25 CS (25% a.i), Marshal 400 SC (40.3% a.i.) and Marshal 35 STD (35% a.i.) were assessed. The conclusions of these studies were that Marshal 25 STW is minimally irritating to the eye, Marshal 35 STD is mildly irritating to the eye and that the other formulations do not induce ocular irritation (Freeman, 1985d, 1989d; Pels Rijcken, 1991a; Klein, 1993d; Mello dos Santos, 1998e). Statements of compliance with GLP and QA were provided.

(c) *Dermal irritation*

For the present evaluation by the Meeting, no studies of dermal irritation with pure or technical-grade carbosulfan were available. Five studies on dermal irritation with formulations of carbosulfan were performed. The level of active ingredient in these formulations ranged from 25 to 40.3%.

In the studies of dermal irritation in rabbits, performed in compliance with guideline 81-5 or OECD 404, the irritating properties of Marshal 25 STW (25% a.i), Marshal 25 ST (28.9% a.i.), Marshal 25 CS (25% a.i), Marshal 400 SC (40.3% a.i.) and Marshal 35 STD (35% a.i.) were assessed. The conclusions of these studies were that Marshal 25 ST is minimally irritating to the skin, Marshal 400 SC is slightly irritating to the skin, and that the other formulations do not induce dermal irritation (Freeman, 1985e, 1989e; Pels Rijcken 1991b; Klein, 1993c; Mello dos Santos, 1998f). Statements of compliance with GLP and QA were provided.

(d) *Dermal sensitization*

For the present evaluation by the Meeting, no studies of sensitization with pure or technical-grade carbosulfan were available. Four studies on the skin-sensitizing properties of formulations of carbosulfan were performed. The level of active ingredient in these formulations ranged from 25 to 40.3%.

In three standard Buehler assays for skin sensitization, performed according to guideline 81-6, the sensitizing properties of 0.3 g of Marshal 25 STW (25% a.i), 0.4 g of Marshal 25 ST (28.9% a.i.), and 0.4 g of Marshal 40 DB (40% a.i) were assessed. The formulations were moistened with saline and the above-mentioned quantities were used in induction as well as in the challenge phase. None of the formulations induced dermal sensitization. Statements of compliance with GLP and QA were provided (Freeman, 1984b, 1985f, 1989c).

A study of dermal sensitization in guinea-pigs (three males, two females; strain unknown) that received intradermal applications of Marshal 400 SC (40.3% a.i.) was considered to be inadequate by the Meeting, since this test was not performed according to the currently required guidelines, the number of animals was insufficient to perform a sound statistical evaluation, and the methods and results sections were poorly described (Mello dos Santos, 1998d).

## 2.2 *Short-term studies of toxicity*

### (a) *Dermal administration*

#### *Rabbits*

In a study that complied with EPA guideline 82-2, groups of six male and six female New Zealand white rabbits received carbosulfan (purity, 91.6%) at a dose of 0, 50, 200 or 800 mg/kg bw per day for 21 consecutive days in daily applications, under an occlusive wrapping, to the clipped skin of the back (doses were selected on the basis of a range-finding study). The test material, being a liquid, was applied undiluted to the skin, covering about 5%, 10–15% or 75% of the test site in the groups receiving the lowest, intermediate and highest dose, respectively. Animals in the control group were treated in the same manner, but received no test material. Each day, the test material was removed 6 h after application by wiping the treated skin with a gauze pad moistened with methanol and subsequently rinsing it with tepid tap water. The animals were observed daily for mortality, clinical signs or signs of dermal toxicity. Body weight and food consumption were measured weekly. After 21 days, the rabbits were killed and necropsies were performed. One female in the group receiving the highest dose died on day 3. Data from this animal were not included in the analysis and the animal was replaced. Clinical chemistry was performed before the start of treatment and at termination. Plasma and erythrocyte cholinesterase activities were determined before initiation of the study and on days 10 and 21, during the 6 h period of exposure. Cholinesterase activity in the brain was determined at termination, within 2 h after the end of the exposure. Statements of compliance with GLP and QA were provided.

One male and two female rabbits in the groups receiving the highest dose died during the study. The death of one animal was attributed to bronchopneumonia. The cause of death of the other two animals could not be established, but was not considered to be treatment-related. Some rabbits in the group receiving the lowest dose had soft stools. In the animals in the groups receiving the intermediate and highest doses, diarrhoea was frequently observed. In the latter group, one male and several females displayed decreased limb tone, and nasal discharge was observed in two females. All treatment groups showed slight erythema (incidence was dose-dependent) and oedema. No signs of dermal irritation were observed in the control animals. Body weight and food consumption were not affected. Apart from inhibition of cholinesterase activity, no treatment-related haematological and biochemical changes were observed. Organ weights were not affected. Except for dermal effects at the site of application, no microscopic changes were observed. The effect of carbosulfan on cholinesterase activity is summarized in Table 4.

At the highest dose (800 mg/kg bw), erythrocyte cholinesterase activity appeared to be far less sensitive to treatment with carbosulfan than plasma or brain cholinesterase activity. No consistent sex differences in cholinesterase inhibition were found. On the basis of the statistically significant reduction in erythrocyte cholinesterase activity (22%) in females at the intermediate dose, and the statistically not significant decrease in brain cholinesterase activity (30% and 22% in males and females respectively) at the intermediate dose, the study author considered the NOAEL to be 50 mg/kg bw per day (Goldenthal, 1990a).

The Meeting noted that it was not specified at which time-point during the 6 h period of exposure the blood samples for determination of erythrocyte and plasma cholinesterase

**Table 4. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in rabbits after dermal application**

Substrate		Dose (mg/kg bw)					
		50		200		800	
		Males	Females	Males	Females	Males	Females
Erythrocytes	Day 10	86	91	94	94	94	75
	Terminal	84	88	78*	84	84	72*
Plasma	Day 10	63*	75	38*	25*	25*	38*
	Terminal	60*	80	40*	20*	20*	30*
Brain	Terminal	90	78	78	40*	40*	44*

From Goldenthal (1990a)

<sup>a</sup> As cholinesterase activities varied considerably over time, cholinesterase activity of animals in the treatment groups is expressed as per cent of the value for concurrent controls rather than as per cent of the pretest value

\* Significantly different from control

activity were drawn, and no data on the peak plasma concentrations of carbosulfan are available. Furthermore, it was reported that brain cholinesterase activity was assessed “within 2 h after the end of exposure”. The effects of carbamates being known to be transient, blood and brain concentrations of carbosulfan at this time may be below peak concentrations. Therefore, measurements of plasma, erythrocyte and brain cholinesterase activity in this study may underestimate the level of inhibition induced by carbosulfan. The variability in the assay and the relatively small group size may explain why decreases of 20–30% in cholinesterase activity were often not statistically significant. In a second study by the same author (Goldenthal, 1990b), the lowest-observed-adverse-effect level (LOAEL) was 50 mg/kg bw per day in male rabbits given carbosulfan by dermal application (see below). In view of the potential underestimation of the inhibition of cholinesterase activity and the small number of animals per group in the present study, and taking into account data from the second study by Goldenthal, the Meeting considered that the statistically non-significant reduction (22%) in cholinesterase activity in the brain of females at 50 mg/kg bw (the lowest dose) to be an adverse effect. Therefore, the LOAEL for carbosulfan in this study was 50 mg/kg bw per day.

In a study performed in compliance with EPA guideline 82-2, groups of six male New Zealand white rabbits were given carbosulfan (purity, 91.6%) at a dose of 0, 5, 50 or 100 mg/kg bw per day for 21 consecutive days by daily dermal application, under an occlusive wrapping, to the clipped skin of the back (doses were selected on the basis of a range-finding study). The test material, being a liquid, was applied undiluted to the skin, covering <5%, 5% and 10% of the test site in animals in the groups receiving the lowest, intermediate and highest doses, respectively. Animals in the control group were treated in the same manner, but received no test material. Each day, the test material was removed 6 h after application, by wiping the treated skin with a gauze pad moistened with methanol and subsequently rinsing it with tepid tap water. The animals were observed daily for mortality, clinical signs or signs of dermal toxicity. Body weight and food consumption were measured weekly. After 21 days, the rabbits were killed and necropsies were performed. Clinical chemistry was performed before the start of treatment and at termination. Plasma and erythrocyte cholinesterase activity was determined before the initiation of the study and on days 10 and 21, during the 6 h period of exposure. Cholinesterase activity in one-half brain sections was determined at termination, within 2 h after the end of the exposure. Statements

**Table 5. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in male rabbits after dermal application**

Substrate	Time-point	Dose (mg/kg bw)		
		5	50	200
Erythrocytes	Day 10	81	86	74
	Terminal	93	100	90
Plasma	Day 10	89*	67*	67*
	Terminal	75*	63*	63*
Brain	Terminal	92	77*	69*

From Goldenthal (1990b)

<sup>a</sup>As cholinesterase activities varied considerably over time, cholinesterase activity of animals in the treatment groups is expressed as per cent of the value for concurrent controls rather than as per cent of the pretest value

\* Significantly different from control

of compliance with GLP and QA were provided. No clinical signs of toxicity were observed. Dose-related slight (all treatment groups) to moderate (highest dose) erythema and oedema were observed. Body weight and food consumption were not affected. No treatment-related haematological and biochemical changes were observed. Organ weight was not affected. Except for dermal effects at the site of application, no microscopic changes in the organs were observed. The effects of carbosulfan on cholinesterase activity are summarized in Table 5.

Erythrocyte cholinesterase activity appeared to be far less sensitive to treatment with carbosulfan than plasma or brain cholinesterase activity. On the basis of the statistically significant reduction in brain cholinesterase activity in animals in the group receiving 50 mg/kg bw per day, the NOAEL was 5 mg/kg bw per day (Goldenthal, 1990b).

The Meeting noted that it was not specified at which time-point during the 6 h period of exposure the blood samples for determination of erythrocyte and plasma cholinesterase activity were drawn, and no data on the peak plasma concentrations of carbosulfan are available. Furthermore, it is reported that brain cholinesterase activity was assessed “within 2 h after the end of exposure”. The effects of carbamates being known to be transient, blood and brain concentrations of carbosulfan may be below peak concentrations at this time. Therefore, measurements of plasma, erythrocyte and brain cholinesterase activity in this study may underestimate the level of inhibition induced by carbosulfan.

(b) *Inhalation*

*Rats*

Groups of five male and five female Sprague-Dawley rats received whole-body exposure to air containing liquid droplets of carbosulfan technical (purity, 89.6%) stabilized in epoxidized soybean oil, 6 h per day, for 5 days. Mean actual concentrations to which the animals were exposed were 5.4, 15.6, and 47 µg/l. A control group was exposed to room air. An additional group of five rats of each sex were killed before treatment to establish cholinesterase activity before exposure to the test material. During exposure, the groups of animals were observed regularly for clinical signs. Detailed observations of individual animals were carried out before and after exposure. Body weights were measured before exposure on day 1 and after exposure on day 5. After the exposure on day 5, the animals

were killed and necropsies were performed, and cholinesterase activity was determined in erythrocytes, plasma and brain. Histopathology was not performed. Statements of compliance with GLP and QA were provided.

The mass median aerodynamic diameter (MMAD) ranged from 1.9 to 2.6  $\mu\text{m}$ . Effects were observed in all treatment groups, these effects increasing in incidence and severity with increasing dose. Females tended to be more sensitive to exposure to carbosulfan than males. During exposure, fasciculation was observed in all treatment groups. Animals in the groups receiving the intermediate and highest doses also displayed irregular breathing and decreased activity. Additionally, salivation, lacrimation, tremors and anogenital staining were observed in the group receiving the highest dose. After exposure, anogenital staining, decreased activity, lacrimation and irregular breathing were observed to occur in a dose-dependent manner in all groups. On day 5, body weights in all treatment groups tended to be less than those of controls; this effect reached statistical significance in males at the highest dose. Except for discoloration of the anogenital and nasal area, no treatment-related macroscopic changes in the organs were observed. The effects of carbosulfan on cholinesterase activity are summarized in Table 6.

Erythrocyte cholinesterase activity appeared to be far less sensitive to treatment with carbosulfan than plasma or brain cholinesterase activity. No consistent sex differences in cholinesterase inhibition were found. Owing to the reduction in brain cholinesterase activity and the clinical signs observed during and after exposure in the groups receiving the lowest dose, a no-observed-adverse-effect concentration (NOAEC) could not be identified. The lowest-observed-adverse-effect concentration (LOAEC) was 5.4 mg/l (Whitman, 1990a).

The Meeting noted that the animals were killed within 3 h 20 min after the end of the final exposure. The effects of carbamates being known to be transient, blood and brain plasma concentrations of carbosulfan at this time may be below peak concentrations. Therefore, measurements of plasma, erythrocyte and brain cholinesterase activities in this study may underestimate the level of inhibition induced by carbosulfan.

Groups of 10 male and 10 female Sprague-Dawley rats received whole-body exposure to air containing liquid droplets of carbosulfan technical (purity, 89.6%) stabilized in epoxidized soybean oil, 6 h per day, 5 days per week for 3 weeks. Mean actual concentra-

**Table 6. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in rats exposed by inhalation for 5 days**

Substrate	Dose ( $\mu\text{g/l}$ )					
	5.4		15.6		47	
	Males	Females	Males	Females	Males	Females
Erythrocyte	95	95	92*	90*	94	87*
Plasma	61*	53	60*	42*	49*	37*
Brain	59*	52*	54*	52*	38*	40*

From Whitman (1990a)

<sup>a</sup>% of value for controls

\* Significantly different from control

tions to which the animals were exposed were 0.15, 0.65 and 5.34 µg/l. An additional group of 10 rats of each sex was killed before treatment, to establish cholinesterase activity before exposure. During exposure, the groups of animals were observed hourly for clinical signs. Detailed observations of individual animals were carried out before and after exposure. Body weights were measured before study initiation, weekly at the start of the exposure, and before and after exposure on the last day. Ophthalmological examinations were performed before study initiation and during the last week of exposure. Within 3 h 20 min after the last exposure, the animals were killed and necropsies were performed. Blood and brain tissue were collected for haematology and clinical chemistry, including determination of cholinesterase activity in erythrocytes, plasma and brain. Organs were dissected and weighed, and microscopical examination was performed on a full range of organs and tissues. Statements of compliance with GLP and QA were provided. The MMAD ranged from 1.9 to 2.0 µm. No clinical signs were observed in animals at the lowest and intermediate doses. At the highest dose, fasciculations, decreased activity, irregular and laboured breathing, salivation, lacrimation, and dried nasal discharge, anogenital staining, soft stool were observed during or after exposure. No treatment-related effects on ophthalmology and body weight were observed. Occasional findings on macroscopical and histopathological examinations, on organ weights and on blood urea nitrogen values were considered to be incidental and not related to treatment. The effects of carbosulfan on cholinesterase activity are summarized in Table 7.

Erythrocyte cholinesterase activity appeared to be far less sensitive to treatment with carbosulfan than plasma or brain cholinesterase activity. No consistent sex differences in cholinesterase inhibition were found. Although statistically significant, only small decreases in brain cholinesterase activity were observed at the intermediate dose (7% in males, 11% in females). Moreover, no clinical signs of toxicity were observed in this group. On the basis of the occurrence of clinical signs and inhibition of brain cholinesterase activity in animals at the highest dose, the NOAEC was 0.65 µg/l (Whitman, 1990b).

The Meeting noted that the animals were killed within 5 h 52 min after the end of the final exposure. The effects of carbamates being known to be transient, plasma concentrations of carbosulfan at this time may be below peak concentrations. Therefore, measurements of plasma, erythrocyte and brain cholinesterase activity in this study may underestimate the level of inhibition induced by carbosulfan.

**Table 7. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in rats exposed by inhalation for 5 days**

Substrate	Dose (µg/l)					
	0.15		0.65		5.34	
	Males	Females	Males	Females	Males	Females
Erythrocyte	101	95	100	96	92	91
Plasma	100	81	97	102	64*	63*
Brain	98	98	93*	89*	57*	64*

From Whitman (1990b)

<sup>a</sup>% of value for controls

\* Significantly different from control

### 2.3 Long-term studies of toxicity and carcinogenicity

#### *Mice*

Groups of 100 male and 100 female Charles River CD-1 mice were given diets containing carbosulfan (purity, 94.5–95.6%, with 0.6–2.4% carbofuran) at a concentration of 0, 10, 20, 500 or 2500 mg/kg (equal to 0, 1.3, 2.5, 62 and 320 mg/kg bw per day for males, and 0, 1.5, 3.1, 72 and 337 mg/kg bw per day for females) for 24 months. All animals were observed daily for signs of toxicity, moribundity and mortality. Body weight and food consumption values were recorded weekly for the first 14 weeks and every two weeks thereafter. Water consumption was determined monthly. Haematological and biochemical measurements and urine analyses, including cholinesterase activity, were performed for 10 unfasted mice of each sex per group at the 6, 12, 18 and 24 month sacrifices. Ophthalmoscopic evaluations were performed in all survivors at 24 months. Selected organs were weighed, gross necropsies conducted and a complete list of tissues and organs examined microscopically. A statement of compliance with QA was provided.

There were no measurable treatment-related effects on mortality or survival. Mean body weights were reduced throughout the study for males at 500 and 2500 mg/kg and for females at 2500 mg/kg. For females, body-weight changes at other dietary concentrations were sporadic and not related to treatment. Males at 20 mg/kg, however, had decreased body-weight gains from week 42 to terminal sacrifice at 104 weeks. Food consumption was depressed for males and females at 2500 mg/kg, but only sporadic decreases were noted at other dietary concentrations.

There were no measurable differences with regard to general appearance and behaviour, except for increased eye irregularities at 2500 mg/kg. These included corneal opacity, eccentric pupil, and white, cloudy eyes. There were no treatment-related effects or haematological changes, except for a tendency towards slightly increased segmented neutrophils and decreased lymphocyte counts in males at 2500 mg/kg. There were no demonstrated effects on glucose, blood urea nitrogen, alanine aminotransferase or alkaline phosphatase in either sex at any dose. The effects of carbosulfan on cholinesterase activity are summarized in Table 8. Since the observed inhibition of cholinesterase activity in the treated groups was not dependent on the time of sampling (i.e. 6, 12, 18 and 24 months), the mean cholinesterase activity (expressed as a percentage of the control value) and range of the means for the four time-points are shown in Table 8.

Cholinesterase activity for plasma, erythrocytes and brain tissue was significantly depressed in both males and females at 500 and 2500 mg/kg, at all time-points.

Ophthalmoscopic examinations indicated an increase in punctuate opacities of the iris at 500 and 2500 mg/kg. Females in the group receiving the highest dose were observed to have focal retinal degeneration. An apparent difference of opinion was expressed by two ophthalmologists, regarding the incidence of cataracts in male mice. An independent ophthalmological examination was subsequently performed, the results of which were considered by the JMPR in 1986. It was concluded that there was no evidence for cataractogenesis in the study of carcinogenicity in mice. Therefore, the concern expressed by the Meeting in 1984 with regard to the cataractogenic potential of carbosulfan was alleviated. Special evaluation and concern for the iris, because of compound-related effects in the rat (DeProspo et al., 1982b) did not indicate similar effects in mice.

**Table 8. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in mice after administration in the diet**

Substrate	Dietary concentration (mg/kg)			
	10	20	500	2500
<i>Males</i>				
Erythrocytes	98 (91–104)	92 (89–96)	64 (56–73)*	52 (40–63)*
Plasma	93 (86–100)	95 (86–100)	52 (36–73)*	38 (36–41)*
Brain	100 (89–107)	105 (93–114)	51 (39–57)*	35 (32–43)*
<i>Females</i>				
Erythrocyte	96 (90–100)	93 (88–98)	66 (64–70)*	56 (48–60)*
Plasma	99 (85–109)	96 (79–105)	77 (72–81)*	49 (41–53)*
Brain	101 (98–102)	97 (88–106)	50 (35–79)*	40 (29–61)*

From DeProspro et al. (1982a)

<sup>a</sup>Overall mean % activity, relative to controls (range of means)

\*Significantly different from control

Absolute organ weight changes were variably affected in both males and females, except for decreased spleen weight in females at 500 and 2500 mg/kg. Relative spleen weights were also significantly decreased in females at 500 and 2500 mg/kg. Relative brain weights were significantly increased throughout the study for both sexes at 2500 mg/kg. This is considered a reflection of the significant effects on body weight at the higher doses.

The results of gross and histopathological examinations were essentially unremarkable. The most common findings reported were malignant lymphoma and bronchioalveolar adenoma, which were equally distributed among all groups, except for females at the lowest dose; the latter had a significant increase in the number of metastatic malignant lymphomas of mediastinal and mesenteric lymph nodes, as well as thymus and spleen. Generally, the incidence of malignant lymphomas was highest in females in the control group and in the group receiving the lowest dose. The results of the histopathological examination indicated that the type and incidence of non-neoplastic and neoplastic lesions were normal findings in the mouse and were unrelated to treatment. Carbosulfan was not carcinogenic in mice at dietary concentrations up to and including 2500 mg/kg, equal to 320 and 337 mg/kg bw per day for males and females respectively.

On the basis of the decrease in body weight, the NOAEL was 10 mg/kg, equal to 1.3 mg/kg bw per day (DeProspro et al., 1982a).

### *Rats*

Groups of 90 male and 90 female Charles River CD rats were given diets containing carbosulfan (purity, 94.5–95.6%) at a concentration of 0, 10, 20, 500 or 2500 mg/kg (equal to 0, 0.5, 1.0, 27 and 153 mg/kg bw for males, and 0, 0.6, 1.2, 35 and 213 mg/kg bw for females) for 104 weeks. Carbofuran was present in the technical material at a concentration of 0.6–2.4%. Growth was observed by body-weight changes and data on food consumption that were recorded weekly for the first 14 weeks and every two weeks thereafter. Daily observations were made with respect to behavioural changes and mortality. At periodic intervals throughout the study, haematological, biochemical and cholinesterase analyses were performed on unfasted animals. Urine analysis was conducted on fasted animals.

Eyes were examined at 12, 18 and 24 months. At 6, 12 and 18 months of the study, 10 males and 10 females per group were sacrificed and necropsies were performed. At the termination of the study, all surviving animals were sacrificed and gross pathological and microscopic examinations of tissues and organs were made, and selected organs were weighed. A statement of compliance with QA was provided.

Tremors, laboured breathing and eye-related changes were more frequently observed in animals receiving the diets containing carbosulfan at a concentration of 500 and 2500 mg/kg. Mean body weight and food consumption values of animals at 500 and 2500 mg/kg were significantly lower than control values throughout the study, except for female food consumption, which was comparable to controls. Survival was not apparently affected by treatment. Measurement of haematology parameters at 6, 12, 18 and 24 months demonstrated a compound-related effect at 18 months in males and females at 2500 mg/kg, including a significantly increased leukocyte count (primarily segmented neutrophils), and platelet count, slightly increased reticulocyte count and significantly decreased lymphocyte count. After 24 months, haemoglobin, erythrocyte cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were all lower, although not significantly, in males at the highest dose than in controls. After 18 months, urine analysis showed that males and females at the highest dose had higher concentrations of ketones than did controls. At terminal sacrifice, there was an increase in mononuclear leukocyte infiltrates in the kidney in animals receiving the intermediate and highest doses, and an increase in pigmentation (haemosiderin) in the mediastinal lymph nodes of treated females. All these data are indicative of leukocytosis and toxic neutrophilia. During early phases of neutrophilia, there is a tendency toward acidosis, which is demonstrated by the presence of ketone bodies in the urine. The elevated platelet count is also a reflection of hyperactivity of the bone marrow. However, histopathology of bone marrow, spleen and liver was otherwise unremarkable. Biochemical analyses were generally comparable with those for controls, except for males and females at the highest dose; decreased albumin, total protein and globulin were reported in these animals. Plasma, erythrocyte and brain cholinesterase activity was significantly decreased in males and females at a concentration of 500 or 2500 mg/kg. Significantly increased relative brain, heart, liver and kidney weights in males and females at the intermediate and highest doses were attributed to lower body weights. Absolute spleen, adrenal and thyroid weights were uniformly lower for groups receiving carbosulfan at 500 and 2500 mg/kg, but were not different from those of controls on an organ-to-body weight basis. Carbosulfan produced compound-related effects on the eye, which were described pathologically as focal iris atrophy, iris coloboma and absence of iris tissues in males and females at 500 and 2500 mg/kg, as well as degenerative retinopathy in females at 2500 mg/kg. The atrophy of the iris was attributed, in part, to an extensive anti-cholinesterase effect. There were no treatment-related effects on the eye at 10 or 20 mg/kg. Gross and histopathologic examinations of all tissues, except the eye, revealed no compound-related effects on the incidence or type of neoplastic or non-neoplastic changes. Carbosulfan was not carcinogenic to rats at dietary concentrations of up to and including 2500 mg/kg (equal to 153 and 213 mg/kgbw per day for males and females, respectively, the highest dose tested). The NOAEL was 20 mg/kg, equal to 1 mg/kgbw per day, on the basis of pathology of the eye, clinical signs and cholinesterase inhibition (DeProspero et al., 1982b).

#### **2.4 Genotoxicity**

A range of tests for genotoxicity in vitro and in vivo was performed with carbosulfan or formulations of carbosulfan. A number of these studies were reported in the public

literature. Some were poorly described and did not comply with current guidelines, GLP or QA. Positive effects were reported in a number of studies from public literature. The results of tests for genotoxicity are summarized in Table 9.

A test for sister chromatid exchange in human lymphocytes (Rencüzoğullari & Topaktas, 1996) was deemed to be inadequate, since positive controls were lacking and the test was only performed in the absence of metabolic activation. A study in which the genotoxicity of mixtures of carbosulfan and other substances were tested was not evaluated (Rencüzoğullari & Topaktas, 2000). One study that reported negative effects with carbosulfan in an Ames test and positive effects in a test with *Saccharomyces cerevisiae* D61.M was not evaluated, because only the abstract was available (Wiedenmann et al., 1990).

## 2.5 Reproductive toxicity

### (a) Multigeneration studies

#### Rats

Groups of 15 male and 30 female Charles River CD rats were given diets containing carbosulfan at a concentration of 0, 10, 20 and 250 mg/kg (equivalent to 0, 0.67, 1.3 and 16.7 mg/kg bw per day) for three generations. Two successive litters were reared from each female. General condition and behaviour were observed routinely and individual body weights were recorded throughout the study. The number of pups in each litter was examined and pups were culled to 10 per litter at age 4 days. Individual pup weights were measured on days 0, 4, 7, 14 and 21. Ten male and 10 female F<sub>1</sub>b, F<sub>2</sub>b, and F<sub>3</sub>b weanlings were randomly selected for gross necropsy and tissue collection. The F<sub>1</sub>a and F<sub>2</sub>a litters were discarded at weaning, and the F<sub>1</sub>b and F<sub>2</sub>b litters were used to produce succeeding generations. Weanlings not selected for continuation in the study (F<sub>1</sub>a, F<sub>2</sub>a, F<sub>1</sub>b, F<sub>2</sub>b, F<sub>3</sub>a and F<sub>3</sub>b) were subjected to gross external examination and sacrificed and discarded. A statement of compliance with QA was provided.

Body weights of F<sub>0</sub> males and females at 20 and 250 mg/kg showed initial decreases. These decreases were associated with reduced food consumption, and both recovered to normal levels between week 4 and sacrifice. Body weights of parental males (F<sub>1</sub> and F<sub>2</sub>) receiving the diet containing carbosulfan at 250 mg/kg were consistently lower than those of animals in the corresponding control group. A similar effect was observed in females at 250 mg/kg at sometimes during gestation and lactation, but not during the growth phases. Mating index, gestation index and number of viable fetuses were essentially normal throughout the study, except for F<sub>2</sub>b dams at 250 mg/kg, for which values for these parameters were decreased. Litter size, pup weights and/or pup weight gains for all litter sets in the group receiving carbosulfan at 250 mg/kg were significantly lower at most age intervals between birth and weaning than for the concurrent control group. Neonatal survival at 250 mg/kg was also significantly lower for the first four litters (F<sub>1</sub>a, F<sub>1</sub>b, F<sub>2</sub>a and F<sub>2</sub>b). There were no treatment-related gross or histological changes observed among the F<sub>0</sub>, F<sub>1</sub>b and F<sub>2</sub>b adults or the F<sub>1</sub>b, F<sub>2</sub>b and F<sub>3</sub>b weanlings. Carbosulfan did not have an adverse effect on reproductive performance. Pup weight, litter size, and pup survival was decreased at 250 mg/kg. In parental animals, the NOAEL was 20 mg/kg (equivalent to 1.3 mg/kg bw per day), on the basis of the decreases in body weight. The NOAEL for fetotoxicity was 20 mg/kg (equivalent to 1.3 mg/kg bw per day) on the basis of reductions in litter size, pup weight and pup weight gain. The NOAEL for reproductive toxicity was 250 mg/kg (equivalent to 16.7 mg/kg bw per day, the highest dose tested) (Kehoe & MacKenzie, 1982).

**Table 9. Results of studies of genotoxicity with carbosulfan**

End-point	Test object	Concentration	Purity (%)	Results	Reference
<i>In vitro</i>					
Reverse mutation	<i>Bacillus subtilis</i> H-17 (rec <sup>+</sup> ), M-45 (rec <sup>-</sup> )	0.0005–2 mg/plate	NR	Negative	Jagannath (1979) <sup>f</sup>
Reverse mutation	<i>S. typhimurium</i> . TA98, TA100, TA1535, TA1537, TA1538	0.1–10 µl/plate	93	Negative	Haworth et al. (1980) <sup>a</sup>
Reverse mutation	<i>E. coli</i> . WP2, CM611 <i>S. typhimurium</i> . TA1978, TA1538	0.025, 0.05, 0.1 & 0.2 ml/plate	93	Negative	Haworth et al. (1981) <sup>a,d</sup>
Reverse mutation	<i>S. typhimurium</i> . TA97a, TA98, TA100, TA1535	0.001–5 mg/plate	40.3	Negative	Gava (1988) <sup>a,e</sup>
Gene mutation	Mouse lymphoma L5178Y <i>Tk</i> <sup>+/+</sup>	0.0024–0.032 µl/ml, –S9 0.0056–0.075 µl/ml, +S9	93	Negative	Kirby et al. (1981) <sup>a</sup>
Chromosomal aberration	Human lymphocytes	1 × 10 <sup>-6</sup> to 5 × 10 <sup>-5</sup> dilution (v/v)	NR	Positive	Topaktas & Rencüzoğullari (1993) <sup>f</sup>
Sister chromaid exchange	Human lymphocytes	25, 50, 100 µmol/l	NR	Positive	Rencüzoğullari & Topaktas (1998) <sup>g</sup>
Gene conversion, reverse mutation, aneuploidy	<i>S. cerevisiae</i> D7, D61.M	4.9–980 µmol/l	93	Negative	Stehrer-Schmid & Wolf (1995) <sup>h</sup>
<i>In vivo</i>					
Micronucleus formation	Mouse bone-marrow cells	43.5 & 87 mg/kg bw 20.3, 40.6, & 60.9 mg/kg bw (twice, 24h interval)	93	Negative	Kirkhart et al. (1979) <sup>b,i</sup>
Micronucleus formation	Mouse bone-marrow cells	20.3, 40.6 & 60.9 mg/kg bw (twice, 24h interval)	40.3	Negative	Franco Perina (1998) <sup>a,j</sup>
Micronucleus formation	Mouse bone-marrow cells	29.6, 59.2 mg/kg bw	93	Positive	Stehrer-Schmid & Wolf (1995) <sup>k</sup>
Chromosomal aberration	Rat bone-marrow cells	0, 5, 12, 30 mg/kg bw for 5 consecutive days	93	Negative	Putnam & Schechtman (1981) <sup>b,l</sup>
Chromosomal aberrations	Rat bone-marrow cells	12.5, 25, 50 mg/kg bw	NR	Positive	Topaktas & Rencüzoğullari (1996) <sup>m</sup>
Dominant lethal mutation	Mice	0, 7, 20, 60 mg/kg bw for 5 consecutive days	93	Negative	Preache et al. (1981) <sup>a,n</sup>

Positive and negative (solvent) controls were included in all studies

NR, not reported; S9, 9000 × g supernatant of induced rat liver

<sup>a</sup>Statements of compliance with GLP and QA were provided

<sup>b</sup>A statement of compliance with QA was provided

<sup>c</sup>Test substance, FMC 35001. S9 fraction of Aroclor 1254-induced rat liver

<sup>d</sup>Preferential kill of repair-deficient *E. coli* CM611 without liver microsomes, at 0.025–0.2 ml/plate

<sup>e</sup>Test substance was Marshal 400SC. S9 fraction of Aroclor 1254-induced rat liver

<sup>f</sup>Study from the public literature. Both carbosulfan (purity not reported) and a formulation of carbosulfan, identified as Marshal (not further specified) were tested. Chromosomal aberrations were reported at all concentrations of carbosulfan and at the two highest concentrations of Marshal

<sup>g</sup>Study from the public literature. Carbosulfan (purity not reported) induced sister chromatid exchange after 48 h treatment but not after 24 h

<sup>h</sup>Study from the public literature

<sup>i</sup>Test compound was administered orally. A dose of 174 mg/kg bw caused excess mortality and was excluded

<sup>j</sup>Marshal 400SC was administered intraperitoneally, in two doses of 75, 50 and 25% of the LD<sub>50</sub>, separated by a 24 h interval. No deaths were reported. The ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCE:NCE) was not affected (at the highest dose, carbosulfan induced a 14% decrease in the PCE:NCE ratio in males)

<sup>k</sup>Study from the public literature. Carbosulfan was administered intraperitoneally. Treatment with carbosulfan resulted in a modest, time-dependent increase in micronucleus formation

<sup>l</sup>Test compound was administered orally in single doses

<sup>m</sup>Study from the public literature. A formulation of carbosulfan, identified as Marshal (not further specified) was administered intraperitoneally. Chromosomal aberrations were reported at all concentrations and time-points

<sup>n</sup>The test compound was administered orally. Parameters evaluated were fertility index, total number of implantations, number of live implantations, number of early and late implantation deaths, body weights and clinical signs

(b) *Developmental toxicity*

*Rats*

Groups of 25 female Charles River CD rats were given carbosulfan at a dose of 0, 2, 10 and 20 mg/kg bw per day by oral gavage on days 6–19 of gestation. Necropsies were performed on surviving females on day 20 and on fetuses delivered by hysterotomy. The number and position of viable/non-viable fetuses, early/late resorptions, mean number of corpora lutea and total number of implantations were recorded. External, internal and skeletal examinations of fetuses were performed. One-third of the fetuses were evaluated for soft-tissue anomalies and the remaining two-thirds for skeletal effects. A statement of compliance with QA was provided.

There was no dose-related increase in mortality in any treated group. Body tremors were reported at 20 mg/kg bw per day after administration of each dose. Clear oral discharge was observed in dams at 10 and 20 mg/kg bw per day. Mean maternal body-weight gains were slightly reduced at 10 mg/kg bw per day and significantly reduced at 20 mg/kg during gestation. There were no differences in number of pregnancies, early/late resorptions, viable fetuses, postimplantation loss or sex distribution. The number of corpora lutea per dam was increased in all treated groups, while the mean fetal body weight was significantly reduced at 10 and 20 mg/kg bw per day. There was also an increase in the number of litters with developmental variations at 20 mg/kg bw per day; these variations included reduced ossification of the skull, unossified hyoid body, unossified sternbrae 5 and 6, and undeveloped renal papilla and/or distended ureters. There were no reported effects on the number or per cent of fetuses or litters with external, internal or skeletal malformations or anomalies at any dose. On the basis of the clinical signs and reduction in body weight, the NOAEL for maternal toxicity was 2 mg/kg bw per day. On the basis of the reduction in fetal body weight, the NOAEL for fetotoxicity was 2 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day (the highest dose tested) (Janes et al., 1980a).

*Rabbits*

Groups of 16 New Zealand albino rabbits were given carbosulfan at a dose of 0, 2, 5, and 10 mg/kg bw per day by gavage on days 6–28 of gestation. Pups were delivered by caesarean section on day 29, and the number, location, and distribution of viable/non-viable fetuses, corpora lutea, early/late resorptions and total implantations were recorded. All fetuses were examined grossly, sectioned for visceral anomalies and stained for skeletal anomalies. A statement of compliance with QA was provided.

No treatment-related effects on appearance and behaviour were observed in the dams. There were three deaths of animals treated at 10 mg/kg, one death in the control group and one death in the group receiving the lowest dose. A cause of death could not be established for two dams in the group receiving the highest dose and in the dam in the group receiving the lowest dose. The deaths of one of the dams at the highest dose and the dam at the lowest dose were attributed to enteritis. The numbers of dams with viable fetuses were 12, 10, 15 and 11 at 0 (control), 2, 5 and 10 mg/kg, respectively. There were no compound-related effects on the number of viable fetuses, corpora lutea, fetal sex distribution or total implantations per dam. There were slight, non-significant increases in postimplantation losses (6, 9, 11 and 13 at 0, 2, 5 and 10 mg/kg bw per day, respectively) and early resorption rate (3, 8, 9 and 11 at 0, 2, 5 and 10 mg/kg bw per day, respectively). Also, the mean fetal body weight was slightly decreased (7%) at the highest dose. There was a single

occurrence of scoliosis in each of the three treated groups, but none was reported in the controls. There were no compound-related effects on skeletal variations, such as delayed ossification. Major vessel variations, identified primarily as left carotid arising from the innominate, were observed in 16.7, 100, 46.7 and 72.7% of the litters at 0, 2, 5, and 10 mg/kgbw per day, respectively (mean of data for historical controls in the test laboratory, 51%). The proportions of fetuses presenting this defect were 4.9, 44, 12.8 and 20.8%, respectively (mean of data for historical controls in the test laboratory, 14%). The increase in major vessel variations was not dose-dependent. Furthermore, the Meeting noted that data for historical controls (MARTA, 2003) show that these major vessel variations are very common and occur at highly variable incidences in rabbits of this strain. Therefore the Meeting considered these effects to be incidental. The NOAEL for maternal and fetotoxicity was 10 mg/kgbw per day (the highest dose tested). Carbosulfan was not teratogenic in rabbits. The NOAEL for developmental toxicity was 10 mg/kgbw per day (the highest dose tested) (Janes et al., 1980b).

## 2.6 *Special studies*

### (a) *Neurotoxicity*

#### (i) *Acute neurotoxicity*

The neurotoxic effects of acute oral exposure to carbosulfan (purity, 88%) were assessed in groups of 27 male and 27 female Crl:CD BR rats treated by gavage in a study that complied with EPA guideline 81-8-SS. The animals received vehicle (corn oil), or carbosulfan at a dose of 0.5, 5 or 30 mg/kgbw. All animals were checked daily for viability and clinical signs. Body weights were measured on days -6, 0, 7, 14 and 15. The doses and time-points were selected on the basis of a preliminary study. Seven animals of each sex per dose were allocated to undergo neuropathological examination on day 15; this consisted of assessment of brain weight and dimensions, evaluation for gross changes, and histopathological examination of a range of central and peripheral nervous system tissues (including eyes, ganglions and nerve fibres). In groups of five animals of each sex per dose, brain, erythrocyte and plasma cholinesterase activities were measured before treatment, and 4 h after dosing on days 0, 7 and 15. Additionally, at termination the brains of animals in these four groups were weighed, and six brain regions (see Table 10) were dissected. The animals that were allocated to undergo neuropathological examination and the animals for which cholinesterase activity was measured on day 15 were also tested in a functional observational battery (FOB) and for locomotor activity before treatment, 4 h after dosing, and on days 7 and 14. Statements of compliance with GLP and QA were provided.

No mortality was observed. During the first week after treatment, body-weight gain was lower in males at the highest dose. Also at the highest dose, some animals had staining of the ventral abdomen and urogenital area on days 1 and 2. Home cage observations revealed one male at the highest dose with tremors on day 0. At 4 h after dosing, body temperatures were decreased in males and females at the highest dose, a few animals with slight tremors and impaired gait were observed during open-field testing, and sensory tests revealed a slow tail-pinch response in males. Furthermore, motor activity was decreased in animals at the highest dose. Effects on brain weight, gross changes and histopathology were considered not to be treatment-related. Data on cholinesterase activity at 4 h after dosing in the groups treated with carbosulfan are summarized in Table 10.

**Table 10. Effects of carbosulfan on cholinesterase activity<sup>a</sup> in rats, 4 h after dosing by oral gavage**

Substrate	Dose (mg/kg bw)					
	0.5		5		30	
	Males	Females	Males	Females	Males	Females
<i>Blood</i>						
Plasma	96	135	76*	116	61*	88
Erythrocytes	101	89	62*	64*	54*	55*
<i>Brain</i>						
Hippocampus	86	108	73	62*	54*	62*
Olfactory region	93	88	66	66*	52*	58*
Midbrain	97	92	64*	61*	53*	53*
Brain stem	84	94	56*	59*	48*	52*
Cerebellum	95	94	74*	67*	61*	68*
Cortex	88*	94	62*	66*	52*	51*

From Knapp (1996)

<sup>a</sup>% of value for controls

\* Significantly different from control

In females, plasma cholinesterase activity appeared to be less sensitive than erythrocyte and brain cholinesterase activity to inhibition by carbosulfan. Brain and erythrocyte cholinesterase activity was reduced in males and females at the intermediate and highest doses, 4 h after administration of carbosulfan. No consistent differences in cholinesterase activity were observed before treatment or on days 7 and 14. Occasional significant differences at these time-points were considered not to be treatment-related.

On the basis of the effects on cholinesterase activity in brain and erythrocytes at the intermediate dose, the NOAEL was 0.5 mg/kg bw (Knapp, 1996).

(ii) *Neurotoxicity after repeated doses*

Groups of 10 male and 10 female Sprague-Dawley CD rats were given diets containing carbosulfan (purity, 88.0%) at a concentration of 0, 20, 1000 or 2000 mg/kg (equal to 0, 1.2, 65 and 131 mg/kg bw per day in males, and 0, 1.4, 79 and 152 mg/kg bw per day in females) for 13 weeks, in a study that complied with EPA guidelines 81, 82 and 83. Clinical signs were recorded daily. Body weights and food consumption were measured weekly. The animals were subjected to FOB and motor activity tests before treatment and 4, 8 and 13 weeks after the start of treatment. At termination, the animals were killed and necropsies were performed. The nervous systems of five animals of each sex in the control group and in the group receiving the highest dose were examined for neuropathological lesions. Statements of compliance with GLP and QA were provided.

At the intermediate and highest doses (dietary concentrations, 1000 or 2000 mg/kg respectively), body weight and body-weight gains were decreased in males and females. At these doses, food consumption in males was decreased throughout the study and food consumption in females was decreased during weeks 2 and 3. Chromodacryorrhoea and decreased faeces were also observed in males at these doses. Additionally, tremors, exophthalmus, chromorhinorrhoea and dehydration were observed in males at the highest dose. In females at the intermediate and highest doses, exophthalmus, tremors, abdominogenital

staining, reddish brown staining of the cage-pan liner, chromorhinorrhoea, chromodacryorrhoea, decreased faeces, dehydration, diarrhoea and unthriftiness were observed. In the FOB, males at the highest dose (2000 mg/kg) showed decrease in foot splay, hindlimb grip strength and urine pools. In females at the highest dose, localized spasms/twitching or tremors, exophthalmos, walking on toes, and slightly impaired gait were observed. A reduction in motor activity was observed in females at the two higher doses at weeks 4 and 13 respectively. No treatment-related effects on necropsy and neuropathological examination were found. Measurements of cholinesterase activity in brain, erythrocyte or plasma were not included in this study. On the basis of the clinical signs, observations in the FOB, and effects on body weight, body-weight gain and food consumption in animals at the two higher doses, the NOAEL was 20 mg/kg, equal to 1.2 mg/kg bw per day (Freeman, 1995).

*(b) Effects of carbosulfan on semen characteristics and serum testosterone*

A study reported in the public literature described the effects of carbosulfan on semen characteristics and serum testosterone concentrations in male rabbits. The study report was confounded by lack of information on the purity of the compound, the doses used, and data on body weight and nutritional state of the animals. Therefore, the Meeting concluded that the significance of the reported effects could not be established (El-Zarkouny et al., 1999).

### Comments

The absorption of radiolabelled carbosulfan administered orally is rapid and almost complete in male and female rats. Elimination is also relatively rapid; most (80–90%) of the absorbed radioactivity is excreted in the urine within 48–72 h, depending on the dose administered. After repeated dosing of rats with carbosulfan, the rate of excretion appeared to be increased (80–87% within 24 h), which may indicate that induction of metabolism has occurred.

Carbosulfan is metabolized by hydrolysis to the 7-phenol or to carbofuran and dibutylamine, and is subsequently further metabolized via hydrolysis, oxidation and conjugation to a variety of metabolites. Metabolites of the dibutylamino moiety may enter the carbon pool and be incorporated into natural constituents of the body. No marked sex-specific differences were observed in rats with regard to the excretion pattern, tissue distribution and metabolite profile of carbosulfan.

Carbosulfan (technical material) is highly toxic when administered orally, with LD<sub>50</sub>s ranging from 90 to 250 mg/kg bw in rats. The LD<sub>50</sub> for carbosulfan was >2000 mg/kg bw in rabbits treated dermally and the LC<sub>50</sub> was 0.61 mg/l in rats treated by inhalation.

Carbosulfan is minimally irritating to the eye, slightly irritating to the skin and is a dermal sensitizer.

In general, in short-term and long-term studies of toxicity, the most sensitive effect of the oral administration of carbosulfan was the inhibition of cholinesterase activity, accompanied at the same or higher doses by clinical signs indicative of cholinesterase inhibition (e.g. salivation, lacrimation, ataxia, tremors, anogenital staining, diarrhoea). In a study of acute oral neurotoxicity in rats, the NOAEL was 0.5 mg/kg bw on the basis of effects on brain cholinesterase activity as measured 4 h after dosing. In a 90-day study in rats, the NOAEL was 20 mg/kg, equivalent to 1 mg/kg bw per day on the basis of inhibition of brain

and erythrocyte cholinesterase activity. In a second 90-day study of rats fed with carbosulfan, the NOAEL was 20 mg/kg, equal to 1.2 mg/kg bw per day, on the basis of clinical signs, observations in FOB and effects on body weight, body-weight gain and food consumption at a dose of 62 mg/kg bw per day. In this study, cholinesterase activity was not determined.

In a 6-month study in dogs, the NOAEL was 50 mg/kg, equivalent to 1.3 mg/kg bw per day, on the basis of effects on blood chemistry parameters and occasional reductions in food consumption and body-weight gain.

In long-term studies in mice and rats, carbosulfan was not carcinogenic at dietary concentrations of up to and including the highest dose tested, 2500 mg/kg, equal to 320 and 153 mg/kg bw per day for mouse and rat, respectively. In the study in mice, the NOAEL was 20 mg/kg, equal to 2.5 mg/kg bw per day, on the basis of reductions in body weight, inhibition of brain and erythrocyte cholinesterase activity and reductions in absolute and relative spleen weight. In the study in rats, the NOAEL was 20 mg/kg, equal to 1 mg/kg bw per day, on the basis of inhibition of brain and erythrocyte cholinesterase activity and pathological changes in the eye, i.e. focal iris atrophy, iris coloboma and absence of iris tissue. The mechanism by which these pathological changes in the eye were induced is not clear.

The genotoxic potential of carbosulfan was investigated in a wide range of tests. Primarily negative results were obtained in a number of tests in vitro and in vivo. Positive effects were observed in a few tests, however these tests were confounded by the use of very high doses in vivo, the occurrence of marked cytotoxicity in vitro and the lack of information on the purity of the test compound. The Meeting concluded that carbosulfan is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice the Meeting concluded that carbosulfan is unlikely to pose a carcinogenic risk to humans.

In a three-generation study of reproductive toxicity, carbosulfan was administered at a dose of 10, 20 or 250 mg/kg of diet. No effects on mating index, gestation index and number of viable fetuses were observed. At 250 mg/kg, pup weight, litter size and pup survival were decreased, as were the body weights of parental males and females at this dose. In parental animals, the NOAEL was 20 mg/kg, equivalent to 1.3 mg/kg bw per day, on the basis of the decreases in body weight. The NOAEL for pup toxicity was 20 mg/kg on the basis of the reductions in litter size, pup body weight and pup body-weight gain. The NOAEL for reproductive toxicity was 250 mg/kg, equivalent to 17 mg/kg bw per day, the highest dose tested.

In studies of developmental toxicity in rats and rabbits, carbosulfan was not teratogenic. The NOAEL for maternal toxicity was 2 mg/kg bw per day in the study in rats, on the basis of clinical signs and reduction in body weight. The NOAEL for toxicity in offspring was 2 mg/kg bw per day, on the basis of the reduction in fetal body weight. In the study in rabbits, the NOAEL for maternal and offspring toxicity was 10 mg/kg bw per day, the highest dose tested.

When tested in hens, carbosulfan did not induce delayed polyneuropathy after a single exposure, according to a study evaluated by the Meeting in 1984.

No new data were available for humans.

The Meeting concluded that the present database is sufficient to characterize the potential hazard of carbosulfan to fetuses, infants and children.

### Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw per day based on a NOAEL of 1 mg/kg bw per day, on the basis of pathological changes in the eye, inhibition of brain and erythrocyte cholinesterase activity and body-weight reduction in the 2-year study in rats, with a safety factor of 100. This safety factor was used because the pathological changes in the eye could not definitely be attributed to inhibition of cholinesterase.

After considering the data available to the present Meeting, as well as the previous evaluations, the Meeting established an acute RfD of 0.02 mg/kg bw. This was based on the NOAEL of 0.5 mg/kg bw per day for inhibition of brain cholinesterase activity in a study of acute neurotoxicity in rats, and a safety factor of 25, as the relevant toxic effects of carbosulfan are dependent on the  $C_{max}$  (Annex 1, reference 95).

#### Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	2-year study of toxicity and carcinogenicity <sup>a</sup>	Toxicity	20 mg/kg, equal to 2.5 mg/kg bw per day	500 mg/kg, equal to 62 mg/kg bw per day
		Carcinogenicity	2500 mg/kg, equal to 320 mg/kg bw per day <sup>c</sup>	
Rat	Three-generation study of reproductive toxicity <sup>a</sup>	Parental and offspring toxicity	20 mg/kg, equivalent to 1.3 mg/kg bw per day	250 mg/kg, equivalent to 17 mg/kg bw per day
		Reproductive toxicity	250 mg/kg, equivalent to 17 mg/kg bw per day <sup>c</sup>	—
	Study of developmental toxicity <sup>b</sup>	Maternal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
		Embryo- and fetotoxicity	2 mg/kg bw per day	10 mg/kg bw per day
	Study of acute neurotoxicity <sup>b</sup>	Neurotoxicity	0.5 mg/kg bw	5 mg/kg bw
	90-day study of neurotoxicity <sup>a</sup>	Neurotoxicity	20 mg/kg, equivalent to 1 mg/kg bw per day	500 mg/kg, equivalent to 25 mg/kg bw per day
2-year study of toxicity and carcinogenicity <sup>a</sup>	Toxicity	20 mg/kg, equal to 1 mg/kg bw per day	500 mg/kg, equal to 27 mg/kg bw per day	
	Carcinogenicity	2500 mg/kg, equal to 153 mg/kg bw per day <sup>c</sup>	—	
Rabbit	Study of developmental toxicity <sup>b</sup>	Maternal toxicity	10 mg/kg bw per day <sup>c</sup>	—
		Embryo- and fetotoxicity	10 mg/kg bw per day <sup>c</sup>	—
Dog	6-month study of toxicity <sup>a</sup>	Toxicity	50 mg/kg, equivalent to 1.3 mg/kg bw per day	500 mg/kg, equivalent to 13 mg/kg bw per day

<sup>a</sup> Diet

<sup>b</sup> Gavage

<sup>c</sup> Highest dose tested

*Estimate of acceptable daily intake for humans*

0–0.01 mg/kg bw

*Estimate of acute reference dose*

0.02 mg/kg bw

*Studies that would provide information useful for continued evaluation of the compound*

Further observations in humans

**Summary of critical end-points for carbosulfan***Absorption, distribution, excretion and metabolism in animals*

Rate and extent of absorption	Rapid and extensive
Dermal absorption	No data (rabbit: reduction in brain cholinesterase activity at 50 mg/kg bw per day)
Distribution	Extensive; highest concentrations in liver, kidney, omental fat, peripheral fat
Potential for accumulation	Low
Rate and extent of excretion	Relatively rapid (80–90% within 48–72 h in rats, mainly in urine)
Metabolism in animals	Major metabolites: 3-OH-7-phenol, carbofuran, 3-OH-carbofuran, 3-keto-7-phenol, 7-phenol, dibutylamine (rat)
Toxicologically significant compounds	Carbosulfan, carbofuran

*Acute toxicity*

Rat, LD <sub>50</sub> , oral	90–250 mg/kg bw
Rabbit, LD <sub>50</sub> , dermal	>2000 mg/kg bw
Rat, LC <sub>50</sub> , inhalation	0.61 mg/l
Rabbit, dermal irritation	A mild irritant
Rabbit, ocular irritation	A mild irritant
Dermal sensitization	Sensitizing (Buehler)

*Short-term studies of toxicity*

Target/critical effect	Inhibition of cholinesterase activity in brain and erythrocytes
Lowest relevant oral NOAEL	1 mg/kg bw per day (rats)
Lowest relevant dermal NOAEL	5 mg/kg bw per day (rabbits)
Lowest relevant inhalatory NOAEC	0.00065 mg/l (rats)

*Genotoxicity*

Negative in most tests; unlikely to be genotoxic

*Long-term studies of toxicity and carcinogenicity*

Target/critical effect	Inhibition of cholinesterase activity in brain and erythrocytes, pathological changes in the eye
Lowest relevant NOAEL	1 mg/kg bw per day (rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans

*Reproductive toxicity*

Reproduction target/critical effect	Reduction of pup weight, litter size and pup survival (in the presence of parental toxicity)
Lowest relevant reproductive NOAEL	1.3 mg/kg bw per day (rats)
Developmental target	Reduction in pup weight (in the presence of maternal toxicity)
	Not teratogenic
Lowest relevant developmental NOAEL	2 mg/kg bw per day (rats)

*Neurotoxicity/delayed neurotoxicity*

Neurotoxicity	Inhibition of cholinesterase activity in brain and erythrocytes, and clinical and behavioural effects associated with cholinesterase inhibition
Lowest relevant oral NOAEL	0.5 mg/kg bw (rats)
Delayed neurotoxicity	Negative

*Medical data*

None

Summary	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, long-term toxicity	100
Acute RfD	0.02 mg/kg bw	Rat, acute neurotoxicity	25

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