Disulfoton

(CAS No: 298-04-4)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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1 Introduction

The present document contains the assessment of the health hazard of disulfoton by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of The Netherlands. The first draft of this document was prepared by L Portengen, M.Sc.(Wageningen University and Research Centre, Wageningen, the Netherlands)*.

The evaluation of the toxicity of disulfoton has been based on the review by American Conference of Governmental Industrial Hygienists (ACG99). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in December 1999, literature was searched in the databases Medline, Toxline, and Chemical Abstracts covering the period of 1966 until December 1999, and using the following key words: disulfoton, disystox, di-syston, thiodemeton, and 298-04-4. Data of unpublished studies were generally not taken into account. Exceptions were made for studies that were summarised and evaluated by international bodies as the Food and Agricultural Organization/World Health Organization (FAO/WHO: Joint Meeting of the FAO Panel of Experts on Pesticides Residues on Food and the Environment and the WHO Expert Group on Pesticides Residues (JMPR)) (FAO92) and the Health Effects Division (HED) of the US Environmental Protection Agency (EPA) as part of its hazard identification assessment review (And00).

In October 2002, the President of the Health Council released a draft of the document for public review. Comments were received from the following individuals and organisations: J Soave (Health and Safety Executive, London, England).

An additional search in Toxline and Medline in May 2003 did not result in information changing the committee's conclusions.

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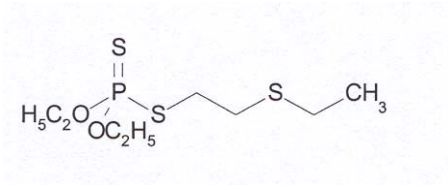
2 Identity

name	:	disulfoton
synonyms	:	phosphorodithioic acid *O*,*O*-diethyl *S*-[2-(ethylthio)ethyl] ester; *O*,*O*-diethyl *S*-2-(ethylthio)ethyl) phosphorodithioate; *O*,*O*-diethyl-*S*-ethylmercaptoethyl dithiophosphate; di-syston
molecular formula	:	$C_8H_{19}O_2PS_3$
structural formula	:	
CAS number	:	298-04-4

3 Physical and chemical properties

molecular weight	:	274.41
boiling point	:	at 0.2 kPa: 132-133°C
melting point	:	-25°C
vapour pressure	:	at 20°C: 0.007 Pa
solubility in water	:	at 20°C: 12 mg/L
log $P_{octanol/water}$	:	4.02
conversion factors	:	not applicable

Data from ACG99, Tom94.

Technical-grade disulfoton is a brown liquid, while the pure substance is an oily, colourless liquid with a characteristic sulphur odour.

Disulfoton is a selective, systemic insecticide and acaricide that is especially effective against sucking insects. Disulfoton is used on a variety of crops including cotton, coffee, tobacco, sugar beets, cole crops, rice, corn, peanuts, wheat, ornamentals, cereal grains, potatoes, and fruit and nut crops (Tom94). According to the database of the Dutch Pesticide Authorisation Board (CTB)*,

* at: http://www.ctb-wageningen.nl/geel.html

disulfoton is at present not registered for its use as an active ingredient in pesticides in the Netherlands.

4 Biotransformation and kinetics

Human data

In the urine of workers, formulating disulfoton, diethyl phosphate (DEP), diethylphosphorothioate (DEPT), and diethylphosphorodithioate (DEPDT) were detected as principal metabolites (Bro81).

Animal data

In a dermal absorption study, labelled disulfoton was applied to the clipped backs (approximately 15cm^2) of male rats (4/group) at 0.85, 8.5, and 85 $\mu g/cm^2$ (approximately 0.05, 0.5, and 5.1 mg/kg bw) for 1, 4, and 10 hours. At termination of exposure, skins were washed and animals were kept for an additional 158 hours to determine the kinetics of absorption and excretion of applied material. A total of about 31-37% of the administered dose was excreted in the urine and 2.7-3.3% in the faeces throughout the 168 hours following dermal application. Ten to 30% of the applied dose evaporated during the 10-hour exposure periods. At the low dose, 5.9%, 13.7%, and 26% were absorbed at 1, 4, and 10 hours following dermal application, at the mid dose, percentages were 4.6, 15.9, and 32.7% and at high dose, 3.6, 12.5, and 25.6%, respectively (War94).

Male and female Sprague-Dawley rats (n=3/sex/group) were given single oral doses of either 0.2 or 1.0 mg 1-ethylen-^{14}C-disulfoton kg/bw. Another 3 rats/sex received 14 daily doses of 0.2 mg/kg unlabelled disulfoton followed by one dose of 0.2 mg/kg bw labelled disulfoton on day 15. The chemical was rapidly absorbed, metabolised, and eliminated under all dosing regimens. Approximately 90% of the radioactivity was recovered in the urine within 24 hours after dosing and excretion was practically completed at 72 hours. Less than 2% of radioactivity was excreted in the faeces, less than 1% exhaled as CO_2 and less than 0.4% retained in tissues and carcasses (Lee85). Disulfoton is biotransformed by oxidation of the thioether moiety into the corresponding disulfoton sulphoxide and disulfoton sulphone and by desulphuration of the P=S moiety to P=O to its disulfoton oxon (demeton-S) analogues. By hydrolysis of these oxidative metabolites, metabolites were identified as sulphonyl [1-

(ethylsulphonyl)-2-(methylsulphinyl)ethane] (43-60% of urine radioactivity) and sulphinyl [1-(ethylsulphinyl)-2-(methylsulphinyl)ethane] (6-20% of urine radioactivity). The committee considers that also the non-labelled metabolites DEPDT, DEPT and DEP are formed and excreted in the urine (Figure1; see Annex I).

In mice, disulfoton was found to bind strongly to cytochrome P450. Since disulfoton also inhibited microsomal ethyl morphine demethylase activity, it was suggested that disulfoton may interfere with the action of mixed function oxidases (Ste74).

5 Effects and mechanism of action

Human data

Apart from a suicidal attempt by a 75-year-old woman, the committee did not find reports on cases of intoxication of human subjects using disulfoton. After ingestion of a large, not further specified, quantity of granular disulfoton, the woman had been admitted to the hospital having vomiting, complaints of nausea, and muscle fasciculations, and 90 minutes after admission, confusion, severe miosis, and cardiac arrhythmias (Fut95).

Five human volunteers were given a daily oral dose of 0.75 mg of disulfoton for 30 days. Two persons served as controls. No changes were observed in levels of plasma ChE and red blood cell AChE. No further details were provided (Rid72).

Animal data

Irritation and sensitisation

The committee did not find data from irritation or sensitisation studies on disulfoton. In a repeated dermal dose study using rabbits, neither skin irritation nor sensitisation were observed (Flu86).

Acute toxicity

Acute inhalation, dermal, and oral LC_{50} and LD_{50} values in test animals are summarised in Table 1.

Table 1 Summary of acute lethal toxicity studies for disulfoton in experimental animals.

exposure route (duration)	vehiculum	species (strain	sex	LC_{50}/LD_{50}	reference
inhalation (1 h)		rat (Wistar)	male	290 mg/m^3	Thy78
(4 h)		rat (Wistar)	male	60 mg/m^3	Thy78
(1 h)		rat (Wistar)	female	63 mg/m^3	Thy78
(4 h)		rat (Wistar)	female	15 mg/m^3	Thy78
		rat	not specified	200 mg/m^3	NIO02
dermal	xylene	rat (Sherman)	male	25	Gai69
	xylene	rat (Sherman)	female	6	Gai69
(24 h)		rat (Wistar)	male	15.9	Mih78
(24 h)		rat (Wistar)	female	3.6	Mih78
		rat (Sprague-Dawley)	male	20	ATS95
		rat	male	22.6	Iya80
		rat	female	7.3	Iya80
		mouse	male+female	35	Iya80
		mouse	not specified	15.6	NIO02
oral	propylene glycol:ethanol	rat (Sprague-Dawley)	male	12.5	Bom58
	propylene glycol:ethanol	rat (Sprague-Dawley)	female	2.6	Bom58
	peanut oil	rat (Sherman)	male	6.8	Gai69
	peanut oil	rat (Sherman)	female	2.3	Gai69
		rat (Hindustan antibiotics)	male	7.2	Paw78
		rat (Hindustan antibiotics)	female	3.2	Paw78
		rat (Wistar)	male	6.2	Mih78
		rat (Wistar)	female	1.9	Mih78
		rat	male	9.6	Iya80
		rat	female	4.2	Iya80
		rat	not specified	10	Sch72
		rat	not specified	2.6	NIO02
		mouse (Hindustan antibiotics)	male	5.8	Paw78
		mouse (Hindustan antibiotics)	female	2.7	Paw78
		mouse (NMRI)	male	7.0	Mih78
		mouse (NMRI)	female	8.2	Mih78
		mouse	male+female	27	Iya80

	mouse (Swiss-Webster)	male	19.3	Ste72
	mouse	not specified	4.8	NIO02
	guinea pig	male	8.9	ATS95
	guinea pig	female	12.7	ATS95
propylene glycol:ethanol	guinea pig	male	10.8	Bom58
	dog	female	ca. 5	Tom94

Induction of cytochrome P450 by pre-treatment with phenobarbital or 3-methylcholanthrene reduced the acute oral toxicity of disulfoton in rats and mice, while pre-treatment with inhibitors of haem or protein synthesis resulted in an increased mortality (Paw78).

In an acute neurotoxicity study, male Sprague-Dawley rats (n=10/group) were given oral doses of 0, 0.25, 1.5, or 5.0 mg/kg bw of disulfoton (purity: 97.8%) and female rats (10/group) 0, 0.25, 0.75, or 1.5 mg/kg bw. All animals were assessed for reactions in a functional observational battery (FOB) and for motor activity measurements at 90 minutes and at days 7 and 14 after dosing. Plasma ChE and red blood cell AChE activity were determined 24 hours after dosing. Female and male rats had muscle fasciculations and decreased activity of plasma ChE (by 30% in females) and red blood cell AChE (by 53% in females) at 0.75 and 1.5 mg/kg bw, respectively. In addition, at the top doses, both sexes had tremors, ataxia, decreased motor and locomotor activity, and decreased activity of plasma ChE (by 52% in females and 25% in males) and red blood cell AChE (by 75% in females and 21% in males). The NOAEL for neurotoxicity and for inhibition of AChE activity is 0.25 mg/kg bw (And00).

Three studies have been reported on the possible delayed neuropathy of disulfoton in hens. In the first study, chickens were tested for leg weakness following a single oral dose of disulfoton. All animals were given atropine to protect against the acute toxic effects of the pesticide and at the highest dose tested (50 mg/kg bw), no mortality was observed. Birds that received 32 mg/kg bw showed leg weakness within 24 hours for a period of up to 13 days. The NOAEL for neurotoxicity was 16 mg/kg bw (Gai69). Twenty adult White Leghorn hens were given oral doses of 30 mg/kg bw disulfoton (purity: 97.8%) on two separate occasions 22 days apart and observed for 42 days. Birds were protected from lethality with atropine and 2-PAM. Negative control birds were dosed either with atropine/2-PAM without disulfoton (n=5), or not treated (n=5), and positive controls (n=10) were given 500 mg/kg tri-*o*-cresol phosphate (TOCP). Cholinergic symptoms (loss of equilibrium, decreased activity, locomotor ataxia) were observed in 14/20 hens, starting soon after dosing but

disappearing within 5 days. Birds treated with TOCP (8/10) showed symptoms between days 12 and 24 which disappeared in 5 hens before termination of the study. Disulfoton-treated birds did not show histological changes suggestive of neuropathy in peripheral nerves or in spinal cord. TOCP-dosed hens showed axonal degeneration in brain and spinal cord. The authors concluded that disulfoton did not induce acute delayed neuropathy (Hix83). In a third study, 18 LSL laying hens were given a single dose of 40 mg/kg bw of disulfoton. Fifteen hens were used as controls. Prior to disulfoton treatment, immediately after treatment, and at day 1 after treatment, the animals were given atropine/2-PAM. No typical signs of disulfoton-induced neuropathy were seen during the 3-week observation period. No hens died, but body weight was slightly decreased. Brain AChE was inhibited by 83% and 59% at 24 and 48 hours after treatment, respectively. The activity of neuropathy target esterase (NTE), a specific enzyme for delayed neurotoxicity, in spinal cord, sciatic nerves, and brain did not change, nor were microscopic abnormalities observed in these tissues (And00). The committee concludes that disulfoton does not cause acute delayed neuropathy in hens.

Short-term toxicity

Wistar rats (n=10/sex/group) were exposed (nose-only) to technical-grade disulfoton (purity: 94.4%) at aerosol concentrations of 0, 0.5, 1.8, or 9.8 mg/m^3, 4 hours/day, for 5 days, and kept under observation for an additional 14 days. Symptoms typical for cholinergic toxicity were observed in all animals of both sexes at 1.8 mg/m^3 and above. There was no mortality except for 9/10 females exposed to 9.8 mg/m^3 (dying between 1-8 days from the beginning of the study). Body weight and gross pathology were not different among groups. After 3-5 exposures to 1.8 mg/m^3 and above, plasma ChE and red blood cell AChE activities were reduced in males by more than 40% and 16 %, respectively. In females, plasma ChE activity was reduced by more than 31% at 0.5 mg/m^3 and above and red blood cell AChE activity by more than 17% at 1.8 mg/m^3 and above. In this 5-day inhalation rat study, the NOAEL for inhibition of red blood cell AChE activity was 0.5 mg/m^3 (Thy78).

A 3-week inhalation study was conducted on Wister rats (n=10/sex/group) exposed to aerosol concentrations of technical disulfoton (purity: 94.4%) of 0, 0.02, 0.1, 0.5, and 3.7 mg/m^3 (6 hours/day, 5 days/week). At the highest dose level, all rats showed cholinergic symptoms and 5 female rats died. Body weight and clinical chemistry and haematology test data remained within normal limits

at all exposure concentrations. Plasma ChE activity was significantly decreased (>30%) at 0.1 mg/m^3 and above in females but in males only at the highest dose level. Red blood cell AChE and brain AChE activities were significantly inhibited (>20%) at 3.7 mg/m^3 in both sexes. Microscopic examination showed inflammation of the respiratory tract and concurrent bone marrow changes at 0.5 mg/m^3 and higher. The 3-week inhalation rat NOAEL was 0.1 mg/m^3 (Thy80).

In another 3-week inhalation study, Fischer 344 rats were exposed (nose-only) to 0, 0.006, 0.07, and 0.7 mg/m^3 of disulfoton aerosol (purity: 97.8%), 6 hours/day, 5 days/week. No deaths, signs of toxicity, or reduction of brain AChE activity were observed at the termination of the study. The 3-week inhalation rat NOAEL was 0.7 mg/m^3, the highest concentration tested (no more data presented, e.g., whether other cholinesterase activities were measured) (Shi88).

In a 13-week inhalation study, Fisher 344 rats (n=12/sex/group) were exposed (nose-only) to aerosol concentrations of disulfoton (purity: 97.8%) of 0, 0.018, 0.16, or 1.4 mg/m^3, 6 hours/day, 5 days/week. No mortality or cholinergic symptoms of toxicity were observed. At 1.4 mg/m^3, significant inhibition of brain AChE (by 29% and 28% in males and females, respectively), red blood cell AChE (not specified), and plasma ChE activity (by 14% and 31% in males and females, respectively) was measured at termination of the study. At 0.16 mg/m^3, at termination, red blood cell AChE and plasma ChE activities were not significantly changed from pre-exposure levels, but brain AChE activity was statistically significantly depressed by 10%. This inhibition was not considered biologically relevant due to the variation noted in this parameter. Upon microscopic examination of terminally derived tissues, only an increased incidence of inflammation of nasal turbinates in male animals exposed to 1.4 mg/m^3 was observed. In this 3-week inhalation rat study, the NOAEL was 0.16 mg/m^3 based on biological significant inhibition of brain AChE at higher exposures (Shi89).

In a dermal study, disulfoton (technical grade; purity: 97.8%) was applied in a Cremophor EL emulsion to the shaven skin of New Zealand White rabbits (n=5/sex/group) at levels of 0, 0.4, 1.6, or 6.5 mg/kg bw/day (uncovered after application; test substance washed off at the end of each exposure period), 6 hours/day, 5 days/week, for 3 weeks. No dermal reaction to repeated dermal application was seen. Cholinergic signs (muscle spasm, dyspnoea, salivation) were observed in both sexes at the highest dose level and all animals died within the first 10 experimental days. At 1.6 mg/kg bw and lower, no mortality, signs of toxicity, or abnormalities in clinical chemistry and haematology tests, gross or

microscopic examination were seen. At 1.6 mg/kg bw/day, plasma ChE, red blood cell AChE, and brain AChE activities were depressed at termination in both females (by 31-44%, 7-33%, and 7-8%, respectively) and males (by 17-24%, 15-19%, and 7-8%, respectively) compared to controls. In this 3-week dermal rabbit study, the NOAEL was 0.4 mg/kg bw, based on inhibition of red blood cell AChE (Flu86).

Oral (gavage) administration of disulfoton (technical grade; purity: 97%) at 2 mg/kg bw/day for 2 weeks caused a 81% decrease in AChE activity in the cerebral cortex of adult male Sprague-Dawley rats (Yag96).

Long Evans rats (n=4) given disulfoton by gavage at 2 mg/kg/day for 2 weeks showed a significant decrease (16-36%) in the density of muscarinic receptors in the cerebral cortex, hippocampus, and striatum, and depression of brain AChE activity (16-32%). Recovery of brain AChE activity was relatively slow when compared to that of muscarinic receptors and ChE activity in peripheral blood lymphocytes (Fit93). Decreases in muscarinic receptor density following disulfoton administration have been associated with the development of tolerance and with impairment in spatial memory (Cos82).

Disulfoton (technical grade; purity: >98.7%) was fed to Fischer 344 rats (n=18/sex/group) for 13 weeks at dose levels equivalent to 0, 0.063, 0.27, or 1.08 mg/kg bw/day for males and 0, 0.071, 0.315, or 1.3 mg/kg/day for females. At the highest dose, increased CNS activity, tremors, muscle fasciculations, perianal staining, increased defecation, decreased forelimb grip strength, decreased motor and locomotor activity, decreased body weight gain, and corneal opacities were observed. At 0.315 mg/kg bw/day, muscle fasciculations and urine staining were observed in females only. Cholinergic signs of toxicity were evident within 2-3 weeks and persisted till the end of the study. Brain AChE, red blood cell AChE, and plasma ChE activities were inhibited at all dose levels in females (by 14-87%, 15-100%, and 13-96%, respectively) and at the mid and top doses in males (by 35-73%, 67-95%, and 31-74%, respectively). In the males of the low-dose group, red blood cell AChE activity was statistically significantly inhibited by 15% in males but brain AChE and plasma ChE activities remained within normal limits. All ophthalmological findings were considered incidental and not related to treatment and no treatment-related differences in gross or microscopic pathology were observed. In this 13-week oral rat study, no NOAEL for inhibition of brain or red blood cell AChE activity could be determined since these activities were affected at the lowest doses tested (She97).

In a special 6-month study designed to establish a NOAEL and LOAEL for ChE inhibition, 35 male and female Fischer 344 rats were given technical-grade disulfoton (purity: 98-99%) at levels equivalent to 0, 0.02 and 0.03 mg/kg bw/day for both sexes and 0.06 and 0.07 mg/kg bw/day, for males and females, respectively. After 2, 4, and 6 months, 10 rats/sex/group were taken for measurement of red blood cell and brain AChE and plasma ChE assays. No changes occurred in body weight and food consumption, and no clinical signs of toxicity were observed. In females, red blood cell AChE activity was significantly inhibited at all dose levels in a dose-related way (3-14%, 11-17%, and 23-29%, respectively), but in males, inhibition (by 10-16%) was measured at 0.06 mg/kg bw/day only. In females, plasma ChE (8-17%) and brain AChE (7-13%) were inhibited at 0.07 mg/kg bw/day only, but no biologically significant inhibition of brain AChE, red blood cell AChE, or plasma ChE was observed in males at any dose level (Chr93). According to EPA, the NOAEL was 0.03 mg/kg/day, based on biological meaningful cholinesterase inhibition (And00). The committee feels, however, that the 3-14% inhibition of red blood cell AChE activity in females at 0.02 mg/kg/day, the lowest level tested, has to be considered as significant. Therefore, the committee could not establish a NOAEL for AChE inhibition in this 6-month oral rat study.

In a one-year study addressing potential ocular and neurological effects, disulfoton (technical grade in corn oil; purity: 97%) was fed to beagle dogs (n=4/sex/group) at dose levels equivalent to 0, 0.015, 0.12, and 0.32 mg/kg bw/day for males and 0, 0.013, 0.094, and 0.28 mg/kg bw/day for females. Cholinesterase activity was measured in plasma, red blood cell, brain, ocular tissues (retina, cornea, ciliary body), and extraocular muscles (lateral and dorsal recti) of the left eye. An ophthalmological and neurological examination and electroretinography were performed pre-treatment, at 6 months, and just prior to termination. No treatment-related effects on neurovisual performance, clinical neurology (task performance), and clinical chemistry and haematology test data or gross or microscopic post-mortem changes were observed. In the high-dose groups, plasma ChE and red blood cell AChE activities were inhibited by 56-63% and 30-91%. In the mid-dose groups, inhibition of plasma ChE and red blood cell AChE activities was 38-46% and 38-40%, respectively. In the low-dose groups, only plasma ChE activity was slightly inhibited (10%) in males after 6 months. Brain AChE activity was depressed in the high-dose group in male and female animals by 32-33% and in the mid-dose group only in females by 22%. ChE activities in the cornea, the retina, and in the ciliary body were also depressed at the mid and top doses. The depression in ChE activity at the lowest

dose in plasma (10%, temporarily), cornea (33%, males only), and ciliary body (15%, males only) was considered equivocal and not related to treatment. In this one-year oral dog study, the NOAEL was 0.013 mg/kg bw, based on inhibition of brain and red blood cell AChE (Jon99).

In a 2-year oral (diet) study, beagle dogs (n=4/sex/group) were given disulfoton (technical grade; purity: 95.7%) at levels equivalent to 0, 0.0125, 0.025, or 0.05 mg/kg bw/day. After 69 weeks, the top dose was subsequently increased to 0.125 mg/kg bw/day during weeks 70-72, and to 0.2 mg/kg bw/day from week 73 to termination. No treatment-related signs of intoxication or changes in general appearance and behaviour, ophthalmoscopy parameters, food consumption, body weight, haematology, clinical chemistry, or organ weights, or upon microscopic examination were observed. A single dog dosed with 0.0125 mg/kg bw of disulfoton developed interstitial nephritis and died at week 93. No inhibition of plasma ChE or red blood cell or brain AChE activity was observed at dose levels ≤0.025 mg/kg/day. At 0.05 mg/kg bw/day, plasma ChE and red blood cell AChE activity were depressed by 50 and 33% in males and 22 and 36% in females, respectively, at week 40. At termination of exposure, at 0.2 mg/kg bw/day, plasma ChE and red blood cell and brain AChE activities were depressed by 65, 58, and 34% in males and 49, 48, and 18% in females, respectively. The NOAEL for red blood cell and brain AChE inhibition in this 2-year oral dog study was 0.025 mg/kg/day (Hof76).

One-week-old White Leghorn chicks (*Gallus domesticus*) were fed doses of disulfoton (purity: 90%) of 0.29 mg/kg bw for 71 days. No adverse effect on growth was noted. After termination, tissues were pooled for analysis of lipid concentrations. Total lipid content in all organs, except liver and sciatic nerves, was decreased. Also, the ratio of phospholipids to total cholesterol was decreased in all nervous tissues except the spinal cord. However, this ratio was increased in liver and kidney (due to a decrease in total cholesterol in these organs). These results were interpreted as an indication of degenerative changes in the brain (Gop79).

The results of these short-term toxicity studies are summarised in Table 2.

Table 2 Summary of short-term toxicity studies for disulfoton.

exposure route	species (strain; sex; number)	dose levels	exposure duration	critical effect[a]	NOAEL	reference
inhalation	rat (Wistar; n=10/sex/group)	0, 0.5, 1.8, 9.8 mg/m^3	5 days	RAChE[b]	0.5 mg/m^3	Thy78
	rat (Wistar; n=10/sex/group)	0, 0.02, 0.1, 0.5, 3.7 mg/m^3	3 weeks	BAChE[c] RAChE	0.1 mg/m^3	Thy80
	rat (F344; ?)	0, 0.006, 0.07, 0.7 mg/m^3	3 weeks	BAChE	0.7 mg/m^3	Shi88
	rat (F344; n=12/sex/group)	0, 0.018, 0.16, 1.4 mg/m^3	13 weeks	BAChE RAChE	0.16 mg/m^3	Shi89
dermal	rabbit (New Zealand White; n=5/sex/group)	0, 0.4, 1.6, 6.5 mg/kg bw/d	3 weeks	BAChE RAChE	0.4 mg/kg bw	Flu86
oral	rat (Sprague-Dawley; male; ?)	2.0 mg/kg bw/d	2 weeks	BAChE	LOAEL: 2.0 mg/kg bw	Yag96
	rat (Long-Evans; ?; n=4)	2.0 mg/kg bw/d	2 weeks	BAChE	LOAEL: 2.0 mg/kg bw	Fit93
	rat (F344; n=18/sex/group)	0, 0.06, 0.27, 1.1 mg/kg bw/d	13 weeks	BAChE RAChE	LOAEL: 0.06 mg/kg bw	She97
	rat (F344; n=35/sex/group)	0, 0.02, 0.03, 0.07 mg/kg bw/d	6 months	BAChE RAChE	LOAEL: 0.02 mg/kg bw	Chr93
	dog (beagle; n=4/sex/group)	0, 0.015, 0.12, 0.32 mg/kg bw/d	1 year	BAChE RAChE	0.015 mg/kg bw	Jon97, Jon99
	dog (beagle; n=4/sex/group)	0, 0.0125, 0.025, 0.05/0.125/0.2 mg/kg bw/d	2 years	BAChE RAChE	0.025 mg/kg bw	Hof76
	chicken (White Leghorn; female; ?)	0.29 mg/kg bw/d	71days	lipids in nerve tissue	LOAEL: 0.29 mg/kg bw	Gop79

[a] RAChE = red blood cell AChE, BAChE = brain AChE.

Long-term toxicity and carcinogenicity

In a 104-wk feeding study, Fisher 344 rats (n=60/sex/group) were given technical-grade disulfoton (purity: 98.1%) at doses equivalent to 0, 0.04, 0.165, or 0.650 mg/kg bw/day. No effect on mortality or on clinical chemistry and haematology test results were seen at any dose. However, at the highest dose, mean body weights and body weight gains were depressed in both males and females. Gross examination showed an increase in relative organ weight of heart,

liver, kidneys, and lung in female rats and brain in both sexes at 0.650 mg/kg bw/day. Corneal vascularity was increased in males and females at this dose, while corneal epithelial hyperplasia and optic nerve degradation were elevated in females only. No microscopic changes in the eyes could be detected at the lower doses. An increased incidence of cystic degeneration of the Harderian gland was seen at the highest dose in males and at 0.165 and 0.650 mg/kg bw/day in females. Since there is no Harderian gland in humans, the significance of these pathological changes is uncertain. Other non-neoplastic changes in females included skin inflammation, ulceration and hyperkeratosis, skeletal muscle atrophy, and granulomatous inflammation of the lungs at the top dose. There was no statistically significant difference in the incidence and in the type or time of onset of neoplasms between controls and disulfoton-treated animals. At termination of the study, a dose-related inhibition in plasma ChE and red blood cell and brain AChE was observed at all doses in both sexes. Plasma ChE and red blood cell AChE activities were inhibited by 11-94% and 19-80%, respectively, in males and by 25-95% and 12-76%, respectively, in females. Brain AChE activities were inhibited by 15%, 53%, and 79% in males and 21%, 53%, and 82% in females at 0.04, 0.165, and 0.650 mg/kg bw, respectively. Consequently, a NOAEL for inhibition of brain and red blood cell AChE activities could not be established in this 2-year oral rat study (Hay85).

In a 108-week feeding study, Crl:CD-1 mice (n=50/sex/group) were given disulfoton (purity: 98.2%) at levels equivalent to 0, 0.15, 0.6, or 2.4 mg/kg bw/day. No treatment-related effects were observed on body weight, food consumption, haematology, or mortality. At termination of the study, gross examination showed enlarged spleen, liver, and lymph nodes in the high-dose group, with greater frequency in females than in males. There was no statistically significant difference in the incidence of malignant lymphomas or any other tumours between disulfoton-treated groups and the control group. At the end of the study, plasma ChE, red blood cell AchE, and brain AChE activities in the high-dose group were inhibited by 79%, 56%, and 44% in males and by 82%, 50%, and 46% in females, respectively. In this 108-week oral mouse study, the NOAEL for inhibition of red blood cell and brain AChE activities was 0.6 mg/kg bw (Hay83).

Based on these results, the committee does not consider disulfoton to be a carcinogenic compound.

Mutagenicity and genotoxicity

Mutagenicity and genotoxicity assays comprised tests for the detection of gene mutations in bacteria, yeast, and mammalian cells *in vitro* and *in vitro* and *in vivo* cytogenicity and other *in vitro* genotoxicity assays.

- *In vitro* tests
  - Gene mutation assays. Tests for reverse mutations in several strains of *S. typhimurium* and in *E. coli* WP2 were positive at concentrations up to 20,000 µg/plate both with and without metabolic activation (Han75, Mor83, Shi79), but negative when tested by EPA up to 5000 µg/plate (And00). Negative results were also found in *S. typhimurium* strains up to 1000 µg/plate and *S. cerevisae* up to 200 µl/well in the presence or absence of metabolic activation (Inu76, Jag81, Kie86, Wat80). Disulfoton induced gene mutations in cultured mouse lymphoma L5178Y cells at concentrations of 40-90 µg/mL in the absence of S9 activation, but no mutagenic activity was seen in the presence of S9 activation up to a cytotoxic dose (And00). Equivocal results were obtained in an *hprt* forward gene mutation assay in cultured Chinese hamster ovary cells (CHO) when tested at concentrations of 0.03-10 µg/mL with and without S9 activation (Yan88). Disulfoton did not induce sex-linked recessive lethal mutations in *D. melanogaster* (Lee83).
  - Cytogenicity assays. In cultured Chinese hamster V79 cells, the chemical did not induce sister-chromatid exchanges (SCE) at doses up to 80 µg/mL in the presence or absence of a metabolic activation system (Che82). Positive SCE results were seen in cultured Chinese hamster ovary (CHO) cells at concentrations up to 100 µg/mL disulfoton in the absence of metabolic activation, but no increased SCE frequency was observed with S9 activation (Put87). In contrast, in another study, a slightly increased incidence of SCEs was found with metabolic activation only (And00).
  - Other genotoxicity assays. Disulfoton did not induce mitotic recombination in *S. cerevisiae* D3 both with and without metabolic activation (Zim84). It increased unscheduled DNA synthesis in cultured WI-38 human lung fibroblasts at doses up to 4000 µg/mL, but failed to do so in the presence of a metabolic activation system (Mit83).

- *In vivo* tests

NMRI mice given 2 oral doses of disulfoton of 6 and 12 mg/kg bw each, did not show an increased incidence of micronuclei in polychromatic erythrocytes (Her81). This test was also negative in Swiss-Webster mice up to a lethal dose (8 mg/kg bw) administered once daily for 2 consecutive days by intraperitoneal injection (And00). Dominant lethal effects were not induced following a single oral dose of 5 mg/kg bw of disulfoton in male NMRI mice (Her80).

Based on these results, the committee concludes that disulfoton has some mutagenic potential *in vitro*, but this is highly dependent on the type of test-system used. In test animals, no genotoxic activity was detected.

Reproduction toxicity

In a 2-generation reproduction toxicity study in Sprague-Dawley rats (n=30/sex/group), both the F0 generation and the F1 offspring (one litter/animal/group) were given technical-grade disulfoton (purity: 99%) at dose levels equivalent to 0, 0.025, 0.10, or 0.45 mg/kg bw/day. Cholinesterase activities were measured in adults during pre-mating (at 8 weeks) and at termination of the study and in pups at postnatal days 4 and 21 in the 2 generations. In the F0 parents, muscle fasciculations, tremors, salivation, body weight reduction, dams with no milk, and decreased maternal care were observed at the high-dose level. In F0 males, plasma ChE, red blood cell, and brain AChE activities were significantly inhibited (both at pre-mating and at termination) at 0.10 mg/kg bw/day and above, and in females at all dose levels. In F1 parents, tremors, decrease in body weight, and dams with no milk were noted at 0.45 mg/kg bw/day. At 0.10 mg/kg bw/day and above, brain AChE activity was significantly inhibited in both sexes. No treatment-related organ weight or histological changes were observed in either F0 or F1 males or females at any dose level. Effects in F1 and F2 pups were inhibition of red blood cell and brain AChE at days 4 and 21 of lactation, treatment-related deaths, and decrease in body weights at 0.45 mg/kg bw/day. Pup deaths were considered to be due to failure of maternal care. Based on brain AChE inhibition, a parental NOAEL could not be established (LOAEL: 0.025 mg/kg bw/day). The NOAEL for reproduction toxicity in this 2-generation oral rat study was 0.10 mg/kg bw/day (AST97).

In an earlier 2-generation study, Sprague-Dawley rats (n=26/sex/group) were fed technical disulfoton (purity: 97.8%) at dose levels equivalent to 0, 0.04, 0.12, or 0.36 mg/kg bw/day. F0 and F1a rats were given the diet for 15 and 13 weeks,

respectively, prior to mating, and the F1b rats were maintained on the diet continuously throughout production of F2 generations. Parental effects included cholinergic signs (tremors during gestation), reduced body weight gain during gestation and lactation, and reduced fertility index at 0.36 mg/kg bw/day. Cholinesterase activities were not determined. The gestation index and length were not different from the controls at any dose level. Effects in pups included reduced growth and survival, reduced litter count, litter weight, and viability and lactation indices, and inhibition of brain AChE activity (by 50-59%) in both generations at 0.36 mg/kg bw. At 0.12 mg/kg bw, embryotoxicity was noted in F2b animals only, and brain AChE activity was depressed in F1a pups by 24-32%. The parental NOAEL was 0.12 mg/kg bw/day, based on signs of toxicity. Based on reduced viability and lactation indices, and reduced brain AChE activity in F2b and F1a pups, respectively, the NOAEL for reproductive effects in this 2-generation oral rat study was 0.04 mg/kg bw/day (Hix86).

In a developmental toxicity study, pregnant New Zealand White rabbits (n=14-22/group, artificially inseminated) were administered technical grade disulfoton in corn oil by gavage at 0, 0.25, 0.86, or 2.7 mg/kg bw/day during days 6-18 of gestation. Since the highest dose level caused mortality and clinical signs of toxicity in the dams, this level was reduced to 2.0 mg/kg bw/day and later to 1.5 mg/kg/day. On day 29 of gestation, the animals were sacrificed. Nine out of 22 animals survived at the top dose, but in the 2 lower dose groups, no treatment-related deaths were observed. Body weight gain during treatment was reduced in animals dosed with 0.86 mg/kg bw and above. The number of implantations, extent of pre- and post-implantation losses, pup weight at birth, and viability of pups were similar to controls for all treated groups. At the top dose, fetal toxicity was observed but survival, development, and growth were unaffected. No treatment-related soft tissue or skeletal anomalies were noted at any dose level. The NOAEL for maternal and developmental toxicity in this oral rabbit study were 0.25 mg/kg and >3 mg/kg bw/day, respectively (Tes82). In another teratology study, technical-grade disulfoton (purity: 98.2%; in polyethylene glycol 400) was fed by gavage to pregnant Sprague-Dawley rats (25/dose level) at dose levels of 0, 0.1, 0.3, or 1.0 mg/kg bw/day during days 6-15 of gestation. On day 21, the animals were sacrificed. No mortality, signs of toxicity, or changes in body weight gain or feed consumption were observed in treated or control groups. On day 15, plasma ChE and red blood cell AChE activities were significantly reduced at 0.3 mg/kg bw/day. Gross examination did not show treatment-related lesions. There were no significant differences in the number of implantations per litter, live, dead, or resorbed fetuses, and the

incidence of soft tissue abnormalities between treated groups and control animals. At 1.0 mg/kg bw/day, fetuses showed incomplete ossification of the intraparietals and sternebrae. In this oral rat study, the NOAEL for maternal toxicity was 0.1 mg/kg bw/day, based on red blood cell AChE inhibition and the developmental NOAEL was 0.3 mg/kg bw/day (Lam83).

6 Existing guidelines

The current administrative occupational exposure limit (MAC) for disulfoton in the Netherlands is 0.1 mg/m^3, 8-hour TWA, with a skin notation.

Existing occupational exposure limits in some European countries and the USA are summarised in Annex II.

7 Health hazard assessment

The health hazard assessment of disulfoton is based mainly on toxicology reviews issued by the Health Effect Division of the United States EPA for reregistration eligibility (And00) and by the FAO/WHO Joint Meeting on Pesticide Residues for recommendation of an acceptable daily intake (FAO92). The toxicity profile in these reviews is obtained mainly from unpublished reports of toxicology studies conducted for registration purposes by the chemical companies manufacturing or marketing the compound.

Workers can be exposed to disulfoton through inhalation of vapour or aerosols or by direct skin contact with a formulation of the compound. No data is available of the percentage uptake of the compound following inhalation. In rats, the dermal absorption of disulfoton ranged from 25-33% of the dose, 10 hours after application. The extent of absorption following oral intake is greater than 90% in the rat. Following oral or dermal absorption, the compound is rapidly metabolised into breakdown products that, in the rat, are excreted for more than 90% of the dose in the urine. No cases of acute intoxications in humans have been reported. In a human volunteer study, oral intake of 0.75 mg/day for 30 days did not produce inhibition of plasma ChE or red blood cell AChE. Since only one dose was tested and no details were given, the committee considered the study inadequate for establishment of a health-based occupational exposure limit.

Based on results of acute lethal toxicity studies in test animals, the committee considers the compound as very toxic after respiratory, dermal, and oral exposure. The compound did not cause neurological changes indicative of acute delayed neurotoxicity. No significant systemic effects have been reported in

short- or long-term toxicity studies in test animals. However, these studies showed inhibition of plasma ChE and of red blood cell and brain AChE in dogs, rabbits, and rats. These cholinesterases have approximately the same sensitivity for inhibition by disulfoton in these species. NOAELs for brain and for red blood cell AChE inhibition were 0.025 mg/kg bw for dogs (2-year oral study), 0.4 mg/kg bw for rabbits (3-week dermal study), 0.16 mg/m^3 for rats (13-week inhalation study) and <0.04 mg/kg bw for rats (2-year oral study), respectively.

Results of *in vitro* mutagenicity tests with disulfoton are conflicting and seem to be dependent on the test system. However, *in vivo* tests (cytogenicity assays in mice) did not show an increased incidence of abnormalities. Carcinogenicity studies in rats and mice did not show a treatment-related increase in tumour incidence. The committee concludes that the positive genotoxic effects of disulfoton were thus not reflected in carcinogenicity. With regard to reproduction toxicity, the committee concludes that there is no evidence of enhanced susceptibility of offspring as compared to adults in two 2-generation reproduction toxicity studies. In a developmental toxicity study in rats, incomplete ossification was only observed at higher doses as compared to those that cause maternal effects.

Based on the above data, the committee concludes that the mechanism of toxicity of disulfoton in mammals is through inhibition of AChE activity in nerve tissue. The committee identifies inhibition of AChE in brain tissue as the most sensitive adverse toxic effect of disulfoton in animal studies, occurring at dose levels that are lower than those that cause other toxic effects. In human beings, for obvious reasons, brain AChE cannot be measured. Instead, red blood cell AChE, being the same molecular target for inhibition by organophosporus pesticide as brain AChE, is used as a surrogate for brain AChE in assessing the human health risk of exposure to disulfoton (Jey94).

The committee did not consider the disulfoton human volunteer study in deriving a health-based recommended occupational exposure limit (HBROEL). Therefore, studies in test animals are used. The committee takes the 13-week inhalation study in rats, with a NOAEL of 0.16 mg/m^3, as a starting point. For extrapolation from rat to man, an overall assessment factor of 9, covering intra- and interspecies variation, is established. Thus, applying this factor of 9 and the preferred value approach, a health-based occupational exposure limit of 0.02 mg/m^3 is recommended for disulfoton.

The committee recommends a health-based occupational exposure limit for disulfoton of 0.02 mg/m^3, as an 8-hour time-weighted average (TWA).

Disulfoton showed a high acute lethal dermal toxicity in rats. A ratio of the dermal LD_{50} and the calculated inhalation LD_{50} of less than 10 is proposed as one of the criteria for assigning a skin notation (ECE98). Since this criterion is met for disulfoton*, the committee recommends a skin notation.

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Annex I

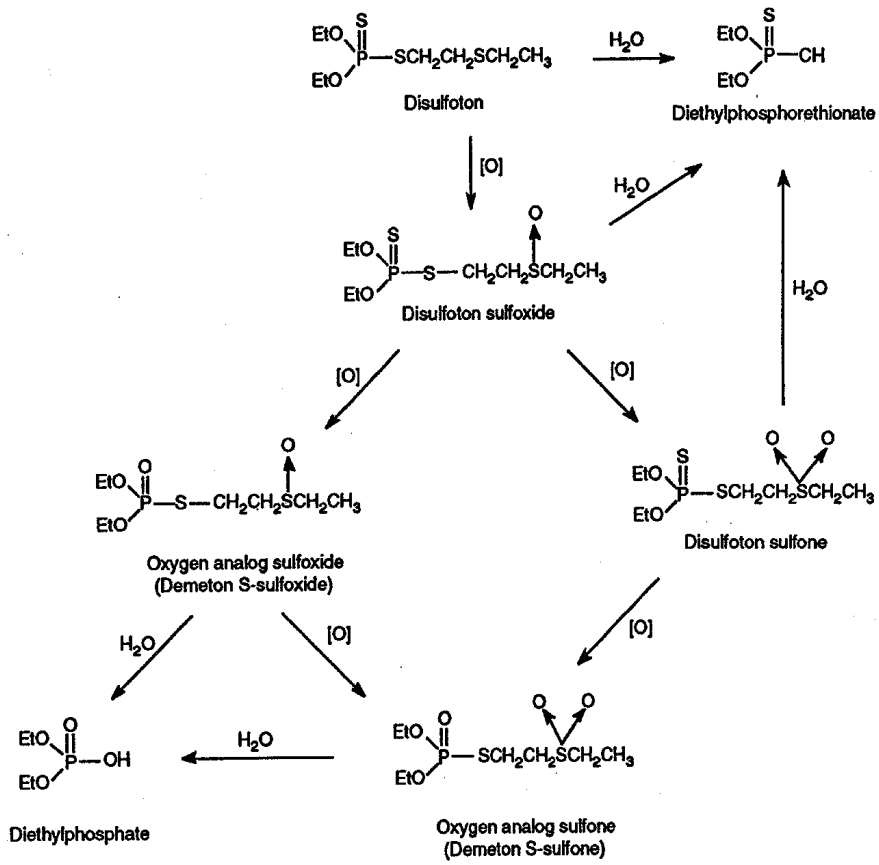

Figure 1 Metabolism scheme for disulfoton (from ATS95).

Annex II

Occupational exposure limits for disulfoton in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	0.1	8 h	administrative	S	SZW03
Germany						
- AGS	-	0.1			S	TRG00
- DFG MAK-Kommission	-	-				DFG02
Great Britain						
- HSE	-	0.1	8 h	OES		HSE02
	-	0.3	15 min			
Sweden	-	-				Arb02
Denmark	-	0.1	8 h		S	Swe00
USA						
- ACGIH	-	0.05[c]	8 h	TLV	S, A4[d]	ACG03b
- OSHA	-	-				ACG03a
- NIOSH	-	0.1	10 h	REL	S	ACG03a
European Union						
- SCOEL	-	-				EC03

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Measured as inhalable fraction of vapour and aerosol.

[d] Classified in carcinogenicity category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.