The MAK Collection for Occupational Health and Safety

Chlorothalonil

MAK Value Documentation, addendum – Translation of the German version from 2018

A. Hartwig[1,*], MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

***** *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords: chlorothalonil; irritation; kidney toxicity; sensitization

Citation Note: Hartwig A, MAK Commission. Chlorothalonil. MAK Value Documentation, addendum – Translation of the German version from 2018. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Jul;4(3):1045-1099]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb189745e6419_w

Republished (online): 08 Aug 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb189745e6419

Addendum completed: 22 Mar 2017

Published (online): 25 Jul 2019

The commission established rules and measures to avoid conflicts of interest.

Chlorothalonil / 2,4,5,6-Tetrachlorobenzene-1,3-dicarbonitrile

MAK Value Documentation

A. Hartwig[1, *], MAK Commission[2, *]

DOI: 10.1002/3527600418.mb189745e6419

Abstract

The German commission for the Investigation of Health Hazards of Chemical compounds in the work Area has re-evaluated chlorothalonil [1897-45-6], considering all toxicological endpoints. Available publications and unpublished study reports are described in detail.

In several carcinogenicity studies, orally applied chlorothalonil causes kidney toxicity in mice and rats and kidney tubular carcinomas only in F344 rats, but not in Osborne-Mendel or Sprague Dawley rats. The reason for this strain difference is unknown. In cells of the kidney proximal tubules thiols are formed which inhibit mitochondrial breathing. This results in cytotoxicity and necrosis followed by cell proliferation, hyperplasia, and tumours. The thiols are generated via β-lyase which is more active in rats than in humans. As the mechanism of the formation of kidney tumours is evaluated as non-genotoxic and no carcinomas occurred in Osborne-Mendel or Sprague Dawley rats at doses higher than those used in F344 rats, chlorothalonil is no longer classified in Category 3B for carcinogens.

Chlorothalonil is irritating to nose, eyes and throat of workers at about 0.3 to 1.2 mg/m^3 and corrosive to the eye of rabbits. A NOAEC after repeated inhalation in humans or animals is not known, therefore no maximum concentration at the workplace (MAK value) can be derived and chlorothalonil is assigned to Section II b of the List of MAK and BAT Values.

Chlorothalonil shows a skin sensitizing potential in humans and animals and labelling with "Sh" (for substances which cause sensitization of the skin), but not with "Sa" (for substances which cause sensitization of the airways) is retained.

Dermal absorption of chlorothalonil is low and does not contribute significantly to systemic toxicity.

Keywords

chlorothalonil; 1,3-dicyanotetrachlorobenzene; 2,4,5,6-tetrachlorobenzo-1,3-dinitrile; 2,4,5,6-tetrachloroisophthalonitrile; 2,4,5,6-tetrachloro-3-cyanobenzonitrile; tetrachloroisophthalonitrile; tetrachloroisophthalodinitrile; m-tetrachlorophthalodinitrile; m-tetrachlorophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

Author Information

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

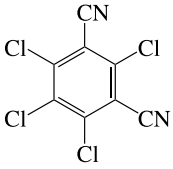
[*] Email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

1046

Chlorothalonil

[1897-45-6]

Supplement 2018

MAK value	**not yet established, see List of MAK and BAT Values, Section II b**
Peak limitation	**–**
Absorption through the skin	**–**
Sensitization (1992)	**Sh**
Carcinogenicity	**–**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	1,3-dicyanotetrachlorobenzene 2,4,5,6-tetrachlorobenzo-1,3-dinitrile 2,4,5,6-tetrachloro-3-cyanobenzonitrile 2,4,5,6-tetrachloroisophthalonitrile tetrachloroisophthalodinitrile tetrachloroisophthalonitrile m-tetrachlorophthalodinitrile m-tetrachlorophthalonitrile
Chemical name	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
CAS number	1897-45-6
Structural formula	
Molecular formula	$C_8Cl_4N_2$
Molar mass	265.9 g/mol
Melting point	253 °C (ECB 2000)

Vapour pressure at 40 °C	< 0.013 hPa (ECB 2000)
log K_{OW}[1)] at 22 °C	2.9 (ECB 2000)
Solubility at 20 °C	0.96 mg/l water (ECB 2000)
1 ml/m³ (ppm) ≙ 11.0 mg/m³	**1 mg/m³ ≙ 0.09 ml/m³ (ppm)**
Stability in water	$t_{1/2}$ at pH 5 and 50 °C: 62 days $t_{1/2}$ at pH 7 and 50 °C: 14 days $t_{1/2}$ at pH 9 and 50 °C: 0.3 days (no other details; ECB 2000)
Production	chlorination of isophthalonitrile or reaction of tetrachloroisophthaloyl chloride with ammonia and subsequent dehydration with, for example, phosphorous pentoxide (Müller et al. 2011)
Purity	98.9% (Syngenta Crop Protection Inc 2005 a)
Impurities	hexachlorobenzene
Uses	non-systemic fungicide against fungi on the leaves of cereals and potatoes (WHO 2009)

For chlorothalonil there is documentation available from 1992 (documentation "Chlorothalonil" 1993) in which the substance is classified in Category 3B for carcinogens and designated as skin sensitizing, and a supplement from 2000 (supplement "Chlorthalonil" 2000, available in German only) which gives a comprehensive description of the data for skin sensitization. New data now allow the re-assessment of all end points.

Determination of exposure levels

Workers who operated a mechanical tomato harvester on four plantations ("A"–"D") which had previously been sprayed once with chlorothalonil wore watertight latex gloves and approximately half of them a cotton mask over the nose and mouth to protect them against the dust. Each group consisted of 6 to 10 persons, approximately half of whom were women. To determine the exposure, the workers at site "A" wore gauze pads along the collars of their shirts, over the front of their clothes at waist level, over their shoulders, and on their upper arms and forearms. The total exposure was calculated from the concentrations on these pads using an anatomical model for the entire body area. The concentration of chlorothalonil residues was determined at sites "B"–"D" using cotton sleeves and cotton vests, which they wore underneath their normal working clothes. From the exposures of 499.6 µg/h determined at site "A" and 43.4 µg/h at sites "B"–"D", the amount of chlorothalonil that

1) octanol/water partition coefficient

penetrated the clothing was calculated to be 8.7%. The amount of chlorothalonil on the skin of the hands, determined by washing with 400 ml of a 1% dioctyl sodium sulfosuccinate solution, was 1.8 µg/h at sites "B"–"D". The dermal exposure calculated from these exposure data for an 8-hour working day and a body weight of 54.8 kg for female workers was 7.2 µg/kg body weight and day. For the amount inhaled by the workers, the sum of chlorothalonil vapour and aerosol was determined; 0.02 mg chlorothalonil/m^3 was obtained at workplace "A" and 0.005 mg/m^3 at the other workplaces. The contribution of inhalation to the total exposure was in the range of 8% to 28% (Spencer et al. 1991). This study was used to determine exposure only; the workers were neither examined nor were effects recorded.

In 5 female workers (25 to 60 years old), the level of inhalation and dermal exposure to chlorothalonil after time spent in a 25 500 m^3 greenhouse, in which a total of 563 g chlorothalonil had been used over the preceding 38 hours, was determined for a period of 5 days. The women started work in the greenhouse at 8 a.m. on Monday morning; the work consisted of attaching plants to a mossy support structure. They wore protective cotton suits without caps and latex gloves over the cotton gloves. A urine sample was taken before starting work on Monday. Thereafter, 24-hour urine samples were analysed for chlorothalonil. By means of personal sampling, inhalable chlorothalonil particles and vapour were determined on every week day. The geometric mean of all weekday values was 5.6 µg/m^3; during this period the values were between 3.14 and 11.57 µg/m^3. The exposure of the skin of their heads and necks was determined using a pad attached to their heads, that of the unexposed skin by attaching 8 pads under their clothing. The chlorothalonil on the skin of their hands was determined by washing with 150 ml 95% ethanol. The greatest amounts on the skin were found to be 0.82–318.7 µg/cm^2 on the forearm, 0.2–66.5 µg/cm^2 on the arm and 3.88–75 µg/cm^2 on the rear upper thigh, and were thus higher than on the face (Aprea et al. 2002). This study, too, was used to record the exposure only.

The aerosol concentrations of chlorothalonil in the air were calculated for the following different scenarios from a spill lasting 30 seconds: (1) a spill of undiluted formulation onto a dry horizontal metal surface; (2) a spill of undiluted formulation onto a rapidly rotating shaft; and (3) pouring undiluted formulation into a container of water. The aerosol generated in these scenarios was compared with that resulting from atomizing dilute chlorothalonil through two different hydraulic nozzles. The simulated spill scenarios generated aerosol concentrations of between 2.1 and 5.3 ng/l, which were somewhat above the detection limit of 1.7 ng/l and the background level of 2.2 ng/l. The two atomizers produced airborne concentrations of 96 and 354 ng/l. From these values it was estimated that a male worker with a respiratory volume of 29 l/min would inhale 0.32 to 0.78 ng chlorothalonil during a 30 second spill, assuming a 1% transfer efficiency (Wolf et al. 1999). Here also, examination of the workers was not carried out.

1 Toxic Effects and Mode of Action

In rats, about 32% of orally administered chlorothalonil is absorbed and about 10% of the dermally applied amount. There is no information on the amount absorbed via inhalation. In occupationally exposed persons, chlorothalonil is irritating to the

eyes, nose and throat in the concentration range of 0.3 to 1.2 mg/m^3. It is slightly irritating to the skin and highly irritating to the eyes of rabbits, and highly irritating to the airways of rats after short-term inhalation exposure. As a result of the irritation, hyperplasia, hyperkeratosis, papillomas and carcinomas of the squamous epithelium of the forestomach occurred after oral administration in rats and mice; these effects are, however, not relevant to humans because of the different anatomy of the species. Studies with oral administration in rats and mice revealed vacuolar degenerative changes in the kidneys, hypertrophy and hyperplasia in the proximal tubular epithelium, and interstitial fibrosis and adenomas at 40 mg/kg body weight and day and above in male and female rats, and at 100 mg/kg body weight and day in male mice. In two carcinogenicity studies with dietary administration of chlorothalonil, carcinomas in the renal proximal tubular epithelium were found at 175 and 183 mg/kg body weight and day in F344 rats. In spite of the observed renal toxicity, however, no renal tubular carcinomas were observed in either a carcinogenicity study with Osborne-Mendel rats with chlorothalonil doses of up to 506 mg/kg body weight and day or in four carcinogenicity studies with Sprague Dawley rats with doses of up to 750 mg/kg body weight and day, with dietary administration in each case. The renal effects were attributed to thiol compounds metabolized specifically in the kidney cells; these inhibit mitochondrial respiration and induce cell proliferation resulting from cytotoxicity in the epithelial cells of the renal proximal tubules.

In dogs, no tumours were found after oral exposure to 375 mg/kg body weight and day for up to two years.

In most tests, chlorothalonil was not genotoxic, either in vitro or in vivo. The few positive results in tests for DNA strand breaks, chromosomal aberrations and micronucleus formation were accompanied by cytotoxicity and mortality and are therefore regarded as secondary effects.

The substance causes sensitization of the skin of humans, guinea pigs and mice.

At 400 mg/kg body weight and day, chlorothalonil is foetotoxic in mice and rats at maternally toxic doses, but not teratogenic.

2 Mechanism of Action

2.1 Organ specificity

Forestomach

Papillomas and carcinomas of the squamous epithelium of the forestomach occurred in rats and mice of both sexes after long-term oral administration; this is described in the documentation of 1992 (documentation "Chlorothalonil" 1993). Chlorothalonil is an irritant. The retention time of ingested food in the glandless forestomach of rats and mice is longer than in the human stomach, which contains glands. Due to the different anatomy and retention times, therefore, these findings are regarded as species-specific to rats and mice and consequently not taken into consideration when evaluating the carcinogenicity of the substance in humans.

Vacuolar degeneration in the epithelium of the renal proximal tubules is more pronounced after the administration of chlorothalonil with the diet than when it is administered by gavage. The authors suggested that this can be attributed to the

direct irritation of the forestomach epithelium caused by chlorothalonil (Fermenta Plant Protection Company 1988 a). When the substance is administered with the diet, the retention time in the forestomach is therefore longer than when it is administered by gavage.

Kidneys

Studies with oral administration in rats revealed in all treated animals vacuolar degenerative changes in the proximal tubules of the kidneys, which appear to emanate from the rough endoplasmic reticulum of the cisternae (see Section 5.2.2). This vacuolar degeneration is similar to that observed after the administration of hypertonic carbohydrates such as glucose, mannitol, sucrose or dextran, all of which attract water into the cells. It can therefore be assumed that an interruption of osmotic control takes place in the proximal tubular cells. This could be connected with the known inhibition of phosphorylation in the mitochondria caused by metabolites of chlorothalonil, which probably affects the cellular energy state. Investigations have shown that dithiol and trithiol metabolites of chlorothalonil inhibit complex II of mitochondrial respiration, which disturbs the energy flow in the tubular cells and the potassium balance. The mechanism underlying the assumption that carbohydrates and chlorothalonil could have the same effects is, however, not clear (ISK Biotech Corporation 1993 b).

The renal tubular toxicity and renal carcinogenicity found in some of the animals after long-term oral administration is attributed to toxic effects of the thiol compounds metabolized specifically in the kidneys; these compounds are formed from the glutathione conjugates of chlorothalonil and subsequent β-lyase cleavage. The dithiol and trithiol compounds inhibit mitochondrial respiration and as a result of their cytotoxicity induce cell proliferation in the kidneys (Wilkinson and Killeen 1996).

The incidence of renal carcinomas was significantly increased only in Fischer rats, but not in Sprague Dawley or Osborne-Mendel rats. The incidence of renal carcinomas in B6C3F1 and CD1 mice was not increased. Dogs are less sensitive than rodents with regard to renal toxicity, presumably because they absorb less chlorothalonil and consequently fewer thiol compounds are formed (documentation "Chlorothalonil" 1993; WHO 2009; Wilkinson and Killeen 1996). Because the β-lyase activity in human kidney tissue is lower than that in rodents, humans are presumably less sensitive to chlorothalonil. However, data for a quantitative comparison are not available (WHO 2009).

2.2 Irritating and sensitizing effects

As an electrophilic aromatic compound, chlorothalonil, like 2,4-dinitrochlorobenzene, enters into substitution reactions with nucleophilic components of proteins, for example with free thiol residues (Enoch et al. 2009; Natsch et al. 2011; Roberts et al. 2015), as a result of which immunologically processable, exogenous structures are formed.

In a comparative in vitro investigation of the reactivity of such electrophilic substances to a heptapeptide containing cysteine, the reactivity of chlorothalonil was between that of 2,4-dinitrofluorobenzene and 2,4-dinitrochlorobenzene, both of

which are sensitizing and irritating. After 24 hours, chlorothalonil led to the depletion of more than 99.5% of the free heptapeptide, whereby similar amounts of two adducts were formed by the substitution of one and two chlorine residues, respectively (Natsch et al. 2011).

This reactivity with proteins or peptides containing thiols could be the reason for its irritation and sensitization potential.

2.3 Oxidative damage

In isolated rat hepatocytes, the extent of the cytotoxicity and lipid peroxidation caused by chlorothalonil was compared with that of two fungicides (captan, dichlofluanide) known to be effective. After incubation for one hour, chlorothalonil concentrations of 25 to 1000 μM led to a 20-fold increase in the phosphatidylcholine hydroperoxide content compared with that in the controls; with the effective fungicides captan and dichlofluanide, 300-fold and 400-fold increases were observed. Dose-dependent cytotoxicity, determined as the lactate dehydrogenase and extracellular protein content, occurred after 60 minutes incubation with chlorothalonil, which was preceded by lipid peroxidation. The antioxidant α-tocopherol and the cytochrome P450 inhibitor SKF-525A prevented lipid peroxidation and cytotoxicity. These results suggest that not chlorothalonil itself but one or more metabolites are responsible for these effects (Suzuki et al. 2004), which, however, were not characterized in this study.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Oral administration

The absorption, distribution and elimination of chlorothalonil have been investigated in several studies (Table 1 and WHO 2009). In dogs given a single oral dose of 50 mg/kg body weight, about 8% of the dose was absorbed. In rats given single oral doses of chlorothalonil of 1.5 to 50 mg/kg body weight, absorption amounted to 32%. The highest tissue concentration in rats was found in the kidneys, which is probably because of the high binding affinity of the substance to kidney proteins. The maximum concentrations in the blood of Sprague Dawley rats given single gavage doses of 5, 50 or 200 mg ^{14}C-chlorothalonil/kg body weight were found after 6.1, 8.9 and 15.9 hours, respectively, and the calculated half-lives were 6, 7.3 and more than 10 hours, respectively. In rats, 16% to 22% of the dose was eliminated in the bile and 7% to 8% with the urine after single oral chlorothalonil doses of 1.5 to 50 mg/kg body weight. After a single oral dose of 200 mg/kg body weight, only 8% of the dose was eliminated in the bile and 5% with the urine, suggesting saturation of the absorption capacity. Compared with male rats, the females eliminated 20% less of the dose in the bile and 35% more with the urine. Elimination with the urine after oral administration was 5% to 10% of the dose in mice, and 1.4% in dogs. The dogs eliminated 2.4% to 7.4% (on average 5.1%) of the dose in the bile, that is about 27%

Table 1 Data for the oral absorption of chlorothalonil (WHO 2009)

	Rat	Mouse	Dog
Dose	6 ♂ per group, Sprague Dawley, ^{14}C, single, gavage	16 ♂ per group, CD-1, ^{14}C, single, gavage	4 ♂ per group, beagle, ^{14}C
	0, 1.5, 5, 50, 200 mg/kg body weight	0, 1.5, 15, 105 mg/kg body weight	50 mg/kg body weight
	bile duct cannulation	no data	bile duct cannulation
	bile, urine, faeces, blood up to 48 hours, animals examined after 48 hours	4 animals (blood) and urine and faeces examined after 9, 24, 96, 168 hours	urine, faeces, bile, cages washed up to 48 hours, animals examined after 48 hours
Absorption	≤ 50 mg/kg body weight: about 32%	no data	2/4 animals vomited → dose ↓ by 7%–9%, results thus corrected, 4.3%–11.1% (mean value 7.7%)
Distribution	≤ 50 mg/kg body weight: max. concentration in the blood: 1.5 mg/kg body weight: after 2–6 hours, 5 mg/kg body weight: after 4 hours, 50 mg/kg body weight: after 6–10 hours; max. 2% in the tissue 200 mg/kg body weight: max. concentration in the blood: after 24 hours	max. 3% in the tissue, especially kidneys	max. 0.1% in liver and kidneys, 1% in muscle, 0.4% in the blood, 0.2% in the fat
Elimination	faeces: no data	faeces: 97% (24 hours), 85% (24 hours), 75% (96 hours)	faeces: 81%
	bile: ≤ 50 mg/kg body weight: 16%–22%, 200 mg/kg body weight: 8%	no data	bile: 2.4%–7.4% (mean value 5.1%) (max. after 10–14 hours)
	urine: ≤ 50 mg/kg body weight: 7%–8%, 200 mg/kg body weight: 5%	urine: 5%–10%	urine: 1.4% (large number of polar, non-identified metabolites (possibly conjugates with GSH))

Table 1 (continued)

	Rat	Mouse	Dog
Dose	6 ♂ per group, Sprague Dawley, 0, 1.5, 5, 50, 160 mg/kg body weight, 5 days: no difference between single and multiple doses as regards the maximum concentration in the blood or kidneys or elimination in percent		
Dose	8 ♂, 4 ♀, Sprague Dawley, ^{14}C, single, gavage, 5 mg/kg body weight, bile duct cannulation, blood after 6, 24, 48 hours, bile, urine, faeces after 48 hours		
Absorption	about 31% of the dose		
Distribution	max. concentration in the blood 6 hours after administration, in bile 2 hours after administration		
Elimination	after 48 hours: ♂: 50% faeces 21% bile, 7.8% urine ♀: 61% faeces, 16.7% bile, 12% urine		
Dose	10 ♂ per group, Sprague Dawley, ^{14}C, single, gavage, 5, 50, 200 mg/kg body weight; max. blood concentration 6 hours, 9 hours, 15.9 hours; calculated $T_{1/2}$: 6 hours, 7.3 hours, > 10 hours		

of the amount eliminated in the bile in rats. Enterohepatic circulation plays a role in the metabolism and elimination of chlorothalonil. The administration of probenecid, an inhibitor of various transporters in renal cells, decreased the elimination of ^{14}C with the urine in rats by approximately 50% compared with the administration of ^{14}C-chlorothalonil alone. It therefore appears that active transport processes are involved in the elimination of the substance (ISK Biosciences Corporation 1995 a; WHO 2009).

Glutathione S-conjugates formed in the liver of rats were channelled through the canalicular membrane using a high affinity transporter and were effectively eliminated with the bile. In order to improve the detection of the glutathione S-conjugates both quantitatively and qualitatively, rats were treated with the irreversible γ–glutamyltranspeptidase inhibitor acivicin. Within 3 hours after oral administration of a chlorothalonil dose of 50 mg/kg body weight, low concentrations of 4,6-bis(glutathione-S-yl)-2,5-dichloroisophthalonitrile (a maximum 0.1% of the administered chlorothalonil amount) were found in the bile. Unchanged chlorothalonil or 4-hydroxyhydroisophthalonitrile were not present in the bile. In the urine, 2,4-bis(glutathione-S-yl)-2,5-dichloroisophthalonitrile and 2,5,6-tris(glutathione-S-yl)-2,5-dichloroisophthalonitrile were found in concentrations close to the detection limit (Rosner et al. 1996).

Groups of 4 conventional and 4 germ-free Sprague Dawley rats were given a single ^{14}C-chlorothalonil dose of 50 mg/kg body weight by gavage, and the radioactivity recovered in the urine was determined for 4 days. The conventional rats eliminated 5.48% (within 24 hours) and 6.56% (within 96 hours) of the dose; this amounted in general to about 35% more cumulative radioactivity eliminated with the urine than that recovered in the germ-free animals at 3.56% (within 24 hours) or 4.24% (within 96 hours). Most of the radioactivity was found as the dimethylthio and trimethylthio metabolites. On day 5, the animals were given another 50 mg/kg body weight by gavage and anaesthetized after 90 minutes. The radioactivity eliminated with the bile between 2 and 8 hours after administration of the substance amounted to 3.7% of the dose in the conventional rats and 1.7% in the germ-free rats. Here also, primarily dithiol and trithiol metabolites were eliminated, always in slightly higher amounts in the conventional rats than in the germ-free animals. In the conventional animals, also a non-methylated dithiol metabolite was found in the bile. In the gastrointestinal microflora, bacteria with a high level of cys-β-lyase activity are present which can therefore affect the metabolism of the glutathione conjugates that have entered the intestine (Hillenweck et al. 1999).

In a preceding study, low absorption (< 4%) of chlorothalonil was observed also ex vivo in the everted gastrointestinal sac of conventional and germ-free Sprague Dawley rats (Hillenweck et al. 1998). On the other hand, for the structurally similar 2,6-dichlorobenzonitrile, more than 70% was found to be orally absorbed in rats as determined by the recovery of radioactivity in the urine and bile. Why the intestinal absorption of chlorothalonil is so much lower, remains unclear (Hillenweck et al. 1999).

Dermal application

In an in vitro investigation, the dermal absorption of chlorothalonil after non-occlusive application of 7, 0.037 and 0.0046 mg/cm^2 for 24 hours was 0.04%, 6.8% and 16.5% of the applied amount, respectively, for rat skin and 0.02%, 0.05% and 0.28%, respectively, for human skin (WHO 2009). The mean fluxes calculated from the human skin absorption data and the applied amounts were 58, 0.77 and 0.54 ng/cm^2 and hour. The corresponding values for rat skin are at least twice as high at 117, 105 and 31.6 ng/cm^2 and hour.

^{14}C-chlorothalonil was applied occlusively to the shaved dorsal skin of rats for up to 24 hours in the form of a suspension concentrate, either undiluted or diluted with water; for applied amounts of 3.4, 0.036 and 0.0042 mg/cm^2, 0.7%, 2% and 10% of the dose, respectively, was found to be absorbed. The majority of the absorbed radioactivity was eliminated via the bile in the faeces (WHO 2009). From the data for the applied doses and amounts absorbed, mean fluxes of 992, 30 and 17.5 ng/cm^2 and hour, respectively were calculated for rats. On the basis of the previously described in vitro investigations which compared rat skin with human skin, it can be assumed that the flux in human skin for the highest dose of 3.4 mg/cm^2 is half as high as that in rats, and is thus 496 ng/cm^2 and hour. In this case, an absorbed amount of 0.99 mg chlorothalonil would be obtained for the exposure of 2000 cm^2 of skin for one hour.

3.2 Metabolism

Chlorothalonil is first conjugated with glutathione and the diglutathione and triglutathione substituents are subsequently metabolized to N-acetylcysteine, cysteinyl-glycine and S-methyl derivatives (see Figure 1) via mercapturates and cysteine conjugates under the participation of β-lyase.

Chlorothalonil was incubated in vitro with rat liver cytosol, to which reduced glutathione was added. Catalysed by glutathione S-transferase, the metabolite 2,4-bis(glutathione-S-yl)-2,5-dichloroisophthalonitrile with a V_{max} of 196 nmol/mg × min^{-1} and a K_M of 8.5 mM was then formed. In the absence of glutathione S-transferase (without cytosol) the V_{max} was 0.8 nmol/mg × min^{-1} and the K_M was 0.3 mM. Thirty seconds after starting the test, 4-(glutathione-S-yl)-2,5,6-trichloroisophthalonitril was formed as an intermediate, and 90 seconds after starting the test, the only metabolite still detectable was 2,4-bis(glutathione-S-yl)-2,5-dichloroisophthalonitrile (Rosner et al. 1996).

In rats given oral chlorothalonil doses of 0.66 or 2.64 mmol/kg body weight (175 or 702 mg/kg body weight) by gavage or with the diet, only the metabolite 4,6-bis(N-acetylcysteine-S-yl)-2,5-dichloroisophthalonitrile was found in the urine. This made up a small amount of the applied chlorothalonil (about 0.5%), which was eliminated with the urine within 36 hours. Most of the administered chlorothalonil (50%–70%), was eliminated with the faeces as unchanged chlorothalonil within 48 hours. In rats, oral administration of chlorothalonil resulted in a non-dose-dependent increase in the elimination of γ-glutamyl transpeptidase, a sensitive parameter for damage to renal proximal tubules. Histopathological examination revealed only very minor lesions in the renal tubules (Rosner et al. 1996).

The Escherichia coli KAM3 cells containing the plasmid pTEcGST overexpress glutathione S-transferase and grow in the presence of 0.37 mM chlorothalonil in the

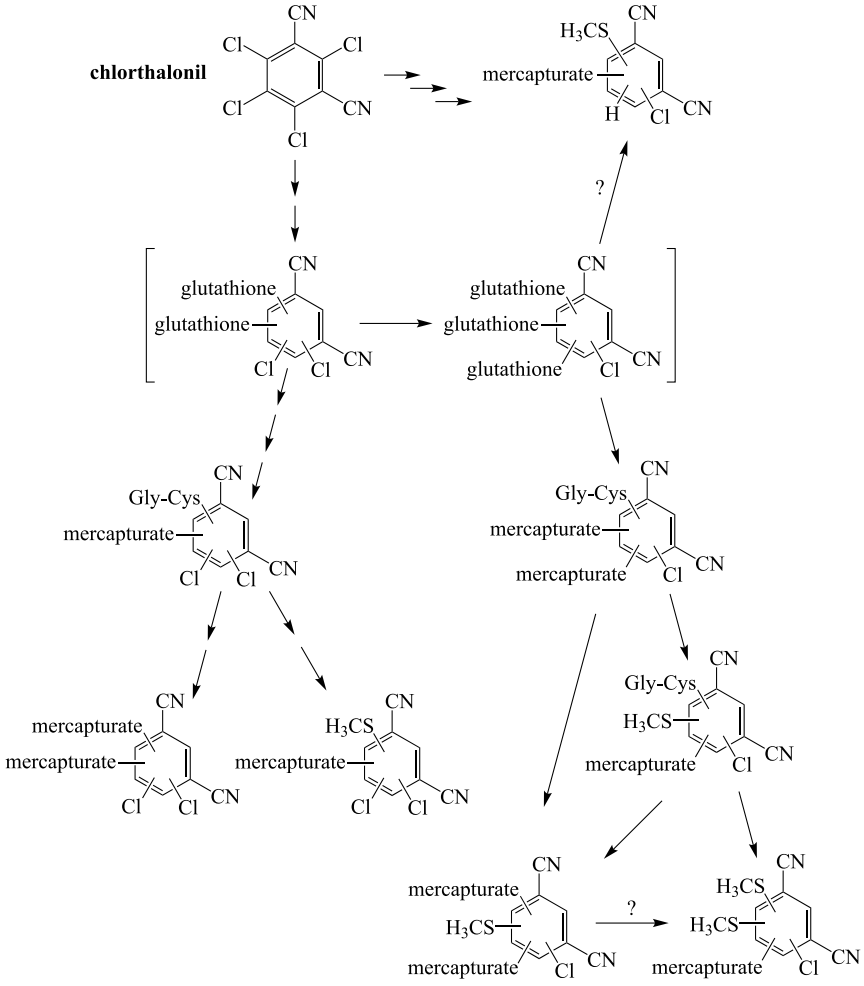


Figure 1 Assumed metabolic pathways of chlorothalonil leading to the formation of the metabolites observed in the urine of the rat (WHO 2009)

medium. The cells containing the vector plasmid only do not grow in the presence of 0.37 mM chlorothalonil. The glutathione S-transferase extracted from Escherichia coli and purified catalysed the reaction of glutathione with chlorothalonil; the reaction product was not identified. The incubation of glutathione with chlorothalonil without glutathione S-transferase produced only a slight reaction. It was concluded from the data that glutathione S-transferase protects the cells from the cytotoxic effects of chlorothalonil (Kim et al. 2004).

To summarize, in view of the demonstrated presence of thiol metabolites in the urine of rats, the β-lyase metabolic pathway may, in principle, be considered as proven. It has, however, not yet been directly proven that this is also the main cause

of the nephrotoxicity; this seems, however, highly probable in the light of investigations of substances metabolized by β-lyase (for example hexachlorobutadiene, tetrachloroethylene) (Wilkinson and Killeen 1996).

4 Effects in Humans

In the concentration range of 0.3 to 1.2 mg/m^3, chlorothalonil was irritating to the eyes, nose and throat and induced coughing, angina and shortness of breath in occupationally exposed persons (Huang et al. 1995).

Allergenic effects

Sensitizing effects on the skin

Since the supplement of 2000 (supplement "Chlorothalonil" 2000, available in German only), only few published clinical data for the skin sensitizing effects of chlorothalonil have become available.

A 58-year-old man developed eczematous skin changes over 6 months while working at a flower auctioneer's after contact with roses imported from Kenia; after this development he was sent to the dermatological clinic. Similar skin changes were found when he was working with amethyst sea holly (Eryngium). Both plants had been treated with agents containing chlorothalonil, but did not produce skin changes in other workers. In patch tests, after 3 and 7 days there were questionable reactions to preparations of 0.1% and 1% chlorothalonil in 2-butanone, but not to 0.001% and 0.01% dilutions. There was also a reaction to a 0.1% preparation in petrolatum which was regarded as questionable, whereas a 0.1% preparation produced a 1+ allergic reaction. The 5 control persons did not react to the test preparations (Lensen et al. 2011).

Of 517 workers employed at the time of the investigation in 4 of 13 banana plantations in northwest Panama, 366 were questioned, among other things, about dermal exposure to pesticides and the protective measures undertaken. In the subsequent examination of the skin, 37 workers were diagnosed with suspected pesticide-related contact dermatitis. These 37 persons and 23 office workers without such a diagnosis underwent patch testing with a series of pesticides containing 16 active ingredients. Of the 37 workers, 15 produced positive reactions, of these in 2 cases also to a preparation containing 0.0017% chlorothalonil in isooctane. Of the 23 symptom-free office workers, none reacted to the chlorothalonil preparation, whereas in 3 persons 2 reactions to benomyl were observed as well as 1 reaction to carbaryl and 1 reaction to thiabendazol (Penagos et al. 2004). Due to the possible irritant effects of isooctane under occlusive conditions it is, however, difficult to attribute the reactions to the chlorothalonil preparation alone.

In a Portuguese trailer tent factory, erythema, conjunctivitis, pruritus and desquamation on the eyelids, face and arms or pain in the throat region occurred in all 11 workers exposed to tent canvas treated with chlorothalonil. In the first patch test, a delayed irritant reaction to extracts from the processed tent material (extraction agent ethanol or acetone; test concentration 10% in petrolatum) as well as to the undiluted finish and to a 10% dilution of the finish was found in most of them, and

also in 5 control persons. As the skin symptoms recurred about 1 year later, also chlorothalonil alone was tested in 4 affected workers. On this occasion, delayed irritant reactions (72-hour reading) to 0.01% chlorothalonil in petrolatum (in 2 of 4 tested persons) and 0.01% chlorothalonil in water (in 3 of 4 tested persons) occurred, but not, however, to 0.01% chlorothalonil in acetone. A reaction to a 1% preparation in acetone was found in 2 of 3 tested persons (Lensen et al. 2007).

In a Japanese study, at the time of the investigation no skin changes could be found in any of 28 workers from a chlorothalonil production plant. Two further workers were not available as they were on holiday at the time of the investigation. The workers had been exposed to chlorothalonil in a 500 m^2 room for about 8 hours per day, 6 days per week, on average for about 3.8 years. Three of the 28 workers reported erythematous, itching skin changes on the neck, hands and the legs, which developed, depending on the workplace, during the summer months. In two of them, but not in any of the remaining 25 or any of 18 workers not exposed, a positive reaction to a 0.05% preparation of chlorothalonil in petrolatum was found in patch tests (Huang et al. 1995).

Another report is available, in which chlorothalonil was suspected of causing photocontact dermatitis. Over a period of 4 years, changes on those parts of the skin exposed to light during the spring and summer season occurred in a man aged 67 years. While gardening he was exposed to a wide range of pesticides, including chlorothalonil. The MED (minimal erythema dose) for UV-A and UV-B was reduced at 2.25 J/cm^2 and 3 mJ/cm^2, respectively. A positive reaction to an aqueous 0.002% chlorothalonil preparation was obtained both in the patch test without exposure to light and with exposure to light; the reaction in the area exposed to light was more pronounced (no other data). Despite avoiding further contact with chlorothalonil his sensitivity to light decreased, but had not completely subsided after 12 months (Matsushita et al. 1996).

Summary: The contact sensitization potential has been confirmed in the more recent investigations.

Sensitizing effects on the airways

Only one report of the sensitizing effects of chlorothalonil on the airways has become available since the supplement of 2000 (supplement "Chlorothalonil" 2000, available in German only).

At a fungicide formulation plant, a 53-year-old worker reported intermittent difficulties in breathing over the past 3 years, which he related to work. The symptoms persisted despite changing to an office job in the same building. Two months before his referral to the clinic he was transferred to another plant on a trial basis. His symptoms improved and his need for bronchodilator inhalation reduced. At the time of the investigation, the non-specific airway reactivity was normal ($PC_{20(histamine)}$ > 16 mg/ml). Serial determinations of the peak expiratory flow (PEF) performed within 6 weeks showed reduced values with diurnal variations of 15% to 40% on work days compared with only 4% to 10% on rest days. In the 30-minute bronchial provocation test with a mixture of chlorothalonil and lactose (12.5 g chlorothalonil in 250 g lactose), starting after about 2–3 hours, a decrease in the forced expiratory one-second volume (FEV1) to a maximum 30% occurred which lasted for about 10 hours. Twenty-four hours after this exposure the $PC_{20(histamine)}$ fell from > 16 mg/ml to 9.0 mg/ml (Draper et al. 2003).

According to a Japanese study, 28 workers at a factory manufacturing chlorothalonil were exposed to chlorothalonil during the whole day in a 500 m^2 room for an average 3.8 years. At the time of investigation, concentrations in the air of between 0.34 and 1.21 mg/m^3 were determined. Compared with the incidence in 18 workers not exposed, an increased frequency of eye, nose and throat irritation (12/13/13 compared with 0/1/2 in the controls), chronic coughing/expectoration/bronchitis (8/8/3 compared with 1/1/1), angina pectoris (9 compared with 0), shortness of breath (6 compared with 0) and rhonchus (wheezing) at the workplace (6 compared with 0) was observed in the exposed persons. While the mean values for the pulmonary function parameters forced vital capacity (FVC), PEF and maximum mid-expiratory flow (MMEF) in both of the groups were not significantly different, the average one-second capacity was markedly reduced (2.29 ± 0.95 l compared with 2.94 ± 0.66 l). No pulmonary function analyses or provocation tests were carried out at the workplace (Huang et al. 1995).

Summary: The available case reports are insufficient to confirm the occurrence of respiratory sensitization.

Carcinogenicity

In a prospective cohort of the "Agricultural Health Study" with 47 625 licenced pesticide applicators living in Iowa and North Carolina, 3657 used chlorothalonil on a median of 3.5 days per year. The exposure took place between 1993 and 1997, and the exposures were recorded by means of a comprehensive questionnaire. The cancer incidence was followed until 31st December, 2004. Chlorothalonil was associated neither with the overall cancer incidence nor with the occurrence of colon, lung or prostate cancer. Only for these cancer sites was a sufficient number of cases available for statistical evaluation (Mozzachio et al. 2008).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The 4-hour LC_{50} in male and female Sprague Dawley rats was 100 mg chlorothalonil/m^3. The MMAD was between 2.5 and 3.6 µm. Gasping and restless behaviour were observed during exposure (purity > 98%, no further data; ISK Biotech Corporation 1993 c; WHO 2009). The very low LC_{50} compared with the toxicity after oral administration is attributed to irritation and the resultant damage to the lungs, such as pulmonary congestion, pulmonary oedema, bronchitis, tracheitis, bronchopneumonia and rhinitis (documentation "Chlorothalonil" 1993). Findings obtained during and after exposure are given in Table 2 and Table 3 (ISK Biotech Corporation 1993 c).

In a safety data sheet, a 1-hour LC_{50} of 310 mg/m^3 is reported for rats (Chem Service 2014).

Table 2 Clinical signs in Sprague Dawley rats during 4-hour exposure to chlorothalonil (ISK Biotech Corporation 1993 c)

Clinical signs*	0 mg/m³ 5 ♂ and 5 ♀	80 mg/m³ 5 ♂ and 5 ♀	140 mg/m³ 5 ♂ and 5 ♀	210 mg/m³ 5 ♂ and 5 ♀
wetness around the eyes	–	4 hours		from 3 hours
eyes partly closed	–	from 1 hour	from 0 hours	from 0.25 hours
gasping	–		4 hours	from 3 hours
exaggerated respiratory movements	–	from 1 hour	from 2 hours	from 0.25 hours
restless behaviour	–	0, 0.25, 0.5 hours: 10/10; 1 hour: 3/10; 2 hours: 1/10; 3 hours: 3/10; 4 hours: 0/10	at 0.5 hours and 1 hour	only during 11 minutes concentration adjustment
discharge from and wetness around snout	–	–	–	4 hours

* examinations at 0, 0.25, 0.5, 1, 2, 3, 4 hours; findings in 10/10 animals, if not otherwise specified

Table 3 Clinical signs in Sprague Dawley rats after 4-hour exposure to chlorothalonil (ISK Biotech Corporation 1993 c)

Clinical signs*	0 mg/m³ 5 ♂ and 5 ♀	80 mg/m³ 5 ♂ and 5 ♀	140 mg/m³ 5 ♂ and 5 ♀	210 mg/m³ 5 ♂ and 5 ♀
mortality		3/10 on day 1 4/10 on day 2	3/10 on day 1 6/10 on day 2	6/10 on day 1 9/10 on day 2
normal behaviour from	–	day 6: 2/10 day 9: 6/10	1/10 on day 4 2/10 on day 5 4/10 on day 6	1/10 on day 11
exaggerated respiratory movements	–	all animals up to 2 hours after exposure, decreasing number, 2/10 on day 8, 0/10 on day 9	1, 6, 6, 6, 4, 2, 2, 2 animals 0 hours, 0.25 hours, 0.5 hours, 1 day, 2 days, 3 days, 4 days, 5 day p.a.	8, 2, 1, 1 animals 1 day, 2 days, 3 days, 4 days p.a.
discharge	–	eyes: directly after the end of exposure: 6/10	nose: directly after the end of exposure: 2/10	nose: 10, 10, 3 animals 0 hours, 0.25 hours, 0.5 hours p.a.
gasping	–	1, 4, 2, 2 animals 2 hours, 1 day, 2 days, 3 days p.a.	8, 3, 3, 1 animals 0 hours, 0.25 hours, 0.5 hours, 1 day	10, 10, 10, 8, 2 animals 0 hours, 0.25 hours, 0.5 hours, 1 day, 2 days p.a.
loud breathing	–	3, 4, 1, 1 animals 2 days, 3 days, 4 days, 5 days p.a.	1, 1 animal 4 days; 5 days p.a.	1 animal each day from day 4 to day 10

* detection directly after end of exposure and after 1 hour, 2 hours and up to 14 days afterwards; p.a. = post applicationem = after administration

5.1.2 Oral administration

In the documentation of 1992 (documentation "Chlorothalonil" 1993), LD_{50} values of 5000 and 15 000 mg/kg body weight are given for rats, of 6000 to 14 522 mg/kg body weight for mice and of 5000 mg/kg body weight for dogs.

These values were confirmed in a study published later. In male and female Sprague Dawley rats, the oral LD_{50} was above 5000 mg chlorothalonil/kg body weight (purity > 98%, administered in aqueous carboxymethyl cellulose; WHO 2009).

After male Fischer 344 rats were given single chlorothalonil doses of 1000 mg/kg body weight in 1% carboxymethyl cellulose by gavage, the animals were examined after 24, 48 and 96 hours, primarily for renal toxicity. A total of four 12-hour urine samples were collected. The levels of glucose, protein and N-acetylglucosaminidase in the urine were increased 2 to 3-fold after 24 and 48 hours, and those of alkaline phosphatase, aspartate aminotransferase and creatine kinase in the plasma were reduced after 24 and 48 hours. After 48 hours, the concentrations of reduced glutathione and cysteine in the kidneys were increased. In the S2 segment of the proximal convoluted tubulus, the numbers of eosinophilic cells (especially after 24 hours), vacuoles (especially after 48 and 96 hours), necrosis in tubular cells and mitosis were increased in a time-dependent fashion. Body and kidney weights were unaffected. As the plasma concentration of creatinine and urea as well as the urine volume and urine excretion were normal, chlorothalonil was concluded to cause mild renal toxicity (Syngenta Crop Protection Inc 2001).

The toxicity of chlorothalonil was investigated in groups of 10 male and 10 female Fischer 344 rats given single gavage doses of 0, 20, 180 or 1000 mg/kg body weight in 1% aqueous carboxymethyl cellulose suspension; the effects on cell proliferation in the kidneys were examined in a range-finding study. After 24 and 96 hours 2 animals per sex and group were used in the toxicity assessment. To evaluate the renal effects, minipumps with bromodeoxyuridine were implanted into the animals 7 days prior to the study; 3 animals per sex and dose were investigated 24 and 96 hours after chlorothalonil administration. Clinical observations, body weights and food consumption were recorded daily, and the urine was collected and analysed on the day of substance administration. All rats underwent gross-pathological examination and the kidneys were examined using light microscopy. No substance-related changes occurred in the examined parameters. This study was available only in the form of a short description (Syngenta Crop Protection Inc 2005 a; WHO 2009).

Cell proliferation in the kidneys was investigated in groups of 5 male and 5 female Fischer 344 rats 24 hours (day 2) or 96 hours (day 5) after gavage administration of chlorothalonil doses of 0, 20, 60 or 250 mg/kg body weight. To assess cell proliferation, the animals were implanted with minipumps which delivered bromodeoxyuridine 7 days prior to the study. There were no clinical signs of toxicity, no consistent effects on food consumption or body weights, and no substance-related effects on clinical-chemical parameters in the urine or on haematological parameters. There were some slight changes in clinico-chemical parameters in the blood; these were isolated or without biological significance, and were therefore regarded as not adverse. No treatment-related effects on kidney weights, kidney pathology and renal cell proliferation occurred (Syngenta Crop Protection Inc 2005 b).

5.1.3 Dermal application

The dermal LD_{50} in male and female Sprague Dawley rats was above 5000 mg chlorothalonil/kg body weight (purity > 98%, administered in water; WHO 2009).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no data available.

5.2.2 Oral administration

In the documentation of 1992 (documentation "Chlorothalonil" 1993) 6 long-term studies in rats and 3 in mice with chlorothalonil and 2 studies with the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile formed in plants and in the soil are described, in which the target organ was primarily the kidneys, but also the (fore-) stomach. The target organs and the type of findings were confirmed in further studies; these are listed in Table 4.

In a 13-week feeding study in CD rats, a dose-dependent decrease in the activity of alanine aminotransferase occurred in all exposed animals. A test with the blood of animals not exposed to chlorothalonil, to which chlorothalonil was added, revealed that no direct interaction between chlorothalonil and blood is responsible for this decrease in activity (Diamond Shamrock Corporation 1981 a).

In carcinogenicity studies the oral NOAEL (no observed adverse effect level) in F344 rats was found to be 1.8 mg chlorothalonil/kg body weight and day. At 3.8 mg/kg body weight and day, the first signs of renal toxicity were chronic progressive nephrosis and a low incidence of mild hyperplasia in the proximal convoluted tubules of female rats (Fermenta ASC Corporation and SDS Biotech KK 1989). In Sprague Dawley rats, the NOAEL for renal toxicity was 3.3 mg/kg body weight and day (LOAEL (lowest observed adverse effect level) 10.6 mg/kg body weight; Vischim Srl 1996). In mice, the oral NOAEL was 5.4 mg/kg body weight and day if the mild irritation of the forestomach is not taken into account (LOAEL 23 mg/kg body weight; Fermenta Plant Protection Company 1987 b). After the administration of chlorothalonil to dogs for one year, the NOAEL was 150 mg/kg body weight and day (LOAEL 500 mg/kg body weight; ISK Biosciences Corporation 1994).

In a 2-year feeding study in dogs, glomerulosclerosis and degenerative changes such as renal tubular hypertrophy and dilation occurred at 375 mg/kg body weight and day and above. Here, the NOAEL for effects on the kidneys was 37.5 mg/kg body weight and day, and the overall NOAEL was 3 mg/kg body weight and day (no other data; WHO 2009).

Table 4 Effects of chlorothalonil after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344, 5 ♂	**1 day, 2 days**, 0, 2× 87.5 mg/kg body weight, 8 hours apart, gavage, technical chlorothalonil in 5% methyl cellulose	kidneys and eyes examined histopathologically 16 hours after final dose; **175 mg/kg body weight**: soft faeces, dry rales, first in the apical region: vacuolar degeneration of proximal renal tubules with solid accumulations, then in the basal region circulating and coalescence of vacuoles without solid accumulations; **2 days**: 2/5 body weights ↓	ISK Biotech Corporation 1993 b
rat, Fischer 344, 3 ♂	**4 days**, 242, 220, 207 mg/kg body weight and day and animal, with the diet, technical chlorothalonil	only kidneys examined histopathologically, food consumption and body weight gains and clinical observations without substance-related effects; **4 days**: widespread vacuolar degeneration of the proximal tubular epithelium (S2 region) in the kidney	Fermenta Plant protection Company 1988 a
rat, Fischer 344, 3 ♂ controls (24 hours, 96 hours), 3 ♂ exposed animals per day	**2, 3, 4 days**, 0, 175 mg/kg body weight and day, gavage, technical chlorothalonil, purity 97.9%	investigation 24 and 96 hours after final dose, no substance-related effects on food consumption, body weight gains or clinical observations, only kidneys examined histopathologically; **2 days**: very sporadic vacuolar degeneration of proximal tubular epithelium of the kidneys in 2/3 animals; **3 days**: vacuolar degeneration in the epithelium of some of the renal proximal tubules (S2 region) (fewer tubules affected than after dietary administration); **4 days**: relative kidney weights ↑ by 9%	Fermenta Plant Protection Company 1988 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344, 6 ♂	**7, 14, 21, 28 days**, 0, 1.5, 15, 175 mg/kg body weight and day, with the diet	organs examined: kidneys (PCNA staining), (fore-)stomach (BrdU staining), duodenum (as control); **15 mg/kg body weight and above**: relative kidney weights ↑, vacuolation of the proximal tubular epithelium on day 28 in 1/6, PCNA index ↑ and PCNA-positive nuclei in cells of the proximal renal tubules, oedema, haemorrhage, erosion, inflammation and hyperplasia of the squamous cell epithelium of the forestomach from day 21, BrdU-positive in the forestomach epithelium on day 28; **175 mg/kg body weight**: vacuolation in the proximal renal tubular epithelium from day 7, forestomach weights ↑, oedema, haemorrhage, erosion, inflammation and hyperplasia of the squamous cell epithelium of the forestomach from day 7, BrdU-positive in forestomach epithelium from day 7	SDS Biotech KK 1996
rat, Sprague Dawley, 25 ♂, 25 ♀	**13 weeks with 13 weeks recovery**, interim examinations after 6 and 13 weeks, 0, 1.5, 3, 10, 40 mg/kg body weight and day, with the diet	**week 13 of exposure**: **1.5 mg/kg body weight and above**: alkaline phosphatase ↓ (♂), absolute and relative kidney weights ↑ (not significant); **3 mg/kg body weight and above**: absolute and relative kidney weights ↑; **10 mg/kg body weight and above**: forestomach: hyperplasia and hyperkeratosis; **40 mg/kg body weight**: alkaline phosphatase ↓, ALT ↓, absolute and relative liver weights ↑ (♂); **recovery**: weeks 19 and 26: **1.5 mg/kg body weight and above**: alkaline phosphatase ↓ (♀)	Diamond Shamrock Corporation 1983 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344, 28 ♂	**1 week, 4, 13 weeks**, 0, 175 mg/kg body weight and day, with the diet, technical chlorothalonil	implantation of a BrdU pump 3.5 days prior to investigation; examination of cell proliferation in kidneys after 7 (14 ♂), 28 and 91 days (7 ♂ each); other parameters: body weights, food consumption, clinical observation, histopathology of kidneys and stomach, cell proliferation in kidneys and duodenum (controls); **175 mg/kg body weight**: body weight gains ↓, relative food consumption ↑, forestomach thickened and with erosions, submucosal oedema, hyperkeratosis and squamous epithelial hyperplasia of the forestomach at all recorded time points, kidney weights ↑, degeneration and hyperplasia in proximally convoluted tubules at all time points; **day 7**: necrosis in tubular epithelium; **days 28 and 91**: tubular hypertrophy, at all time points cell proliferation in kidneys ↑ (maximum after 7 days, then slowly decreasing)	ISK Biosciences Corporation 1996 a
rat, Fischer 344, 15 ♂	**13 weeks**, 0, 75 mg/kg body weight and day or 150 mg **mono-glutathione conjugate**/kg body weight and day, gavage	equimolar doses of chlorothalonil and mono-glutathione conjugate; **exposed animals**: thiol metabolites in the urine, kidney weights ↑; histopathological changes the same in kidneys with both substances: hypertrophy and hyperplasia in the proximal tubular epithelium, vacuolar degeneration, interstitial fibrosis, → similar effects of similar intensity show that glutathione conjugate metabolism is probably responsible for renal toxicity; however: mono-glutathione conjugate does not induce any effects in the forestomach **chlorothalonil**: mucosal thickening and squamous epithelial hyperplasia of the forestomach with ulceration (irritant effects)	Fermenta Plant Protection Company 1987 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, CD, 20 ♂, 20 ♀	**13 weeks**, 0, 40, 80, 175, 375, 750, 1500 mg/kg body weight and day, with the diet, technical chlorothalonil	**40 mg/kg body weight and above**: a number of animals from all dose groups with acute gastritis in the stomach (number inverse to dose: 14/40 at 40 mg/kg body weight, 2/40 at 1500 mg/kg body weight), relative kidney weights ↑ without histopathological correlate, ALT ↓ (demonstrated in 2 different tests, only an increase is regarded as adverse); **175 mg/kg body weight and above**: serum T4 (♂) ↓ (correlated with decreased body weight gains); **375 mg/kg body weight and above**: food intake ↑ (14%, 22%, 34%), body weight gains ↓ (♂: 12%, 23%, 34%; ♀: 10%, 15%, 19%), blood glucose (♂) ↓, blood urea nitrogen ↓, specific gravity of urine (♂) ↑, urine volume (♂) ↓, relative brain weights ↑ (due to body weights), relative heart weights ↑ (due to body weights); **750 mg/kg body weight and above**: soft faeces, mucus in faeces, reduced faeces, red nasal discharge or crusty material around nose, swelling and irritation of anus, poor general condition, blood glucose (♀) ↓, relative liver weights ↑ (due to body weights), relative testis weights ↑ (due to body weights); **1500 mg/kg body weight**: ♀: food intake 12% ↑, serum T4 ↓, relative ovary weights ↑ (due to body weights)	Diamond Shamrock Corporation 1981 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344, 60 ♂, ♀	♂ **27 months,** ♀ **30 months**, 0, 40, 80, 175 mg/kg body weight and day, with the diet, technical chlorothalonil	due to low mortality up to month 20, end of study extended to a maximum 30 months; **40 mg/kg body weight and above**: body weight gains ↓, absolute and relative kidney weights ↑, urea nitrogen and creatinine in the serum ↑, ALT and alkaline phosphatase ↓ (only an increase is regarded as adverse), serum albumin (♂) ↑, irritation of mucous membranes of the oesophagus (hyperplasia and hyperkeratosis) and forestomach (hyperplasia and hyperkeratosis → adenomas and carcinomas and foci of necrosis and ulcers in the squamous epithelium) and glandular stomach and duodenum, chronic glomerulonephritis, tubular cysts, preneoplastic hyperplasia and proliferative damage in the renal tubules (→ tumours); **80 mg/kg body weight and above**: mean body weights ↓ (end of study 10% (♂) 12% (♀)), number of leukaemia cases ↓, serum urea (♂) ↑ (as a result of renal findings); **175 mg/kg body weight**: from month 24 mortality (♂) ↑, dark yellow urine up to month 18, food consumption ↑, mean body weights ↓ (after 12 months 12% (♂) 10% (♀), after 24 months 20% (♂) 15% (♀), end of study 29% (♂) 25% (♀)), serum creatinine (♂) ↑ (as a result of renal findings), serum albumin (♀) ↑, foci of necrosis and ulcers in the squamous epithelium of the glandular stomach	SDS Biotech Corporation 1985 c

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344, 65 ♂, 65 ♀	♂ **23–26 months,** ♀ **29 months**, 0, 1.8, 3.8, 15, 183 mg/kg body weight and day, with the diet, technical chlorothalonil, purity 98.3%	10 animals/sex/group examined after 1 year, all ♂ in 175 mg/kg body weight group after 23 months, all other ♂ examined after 26 months, all ♀ after 29 months, no substance-related findings in ophthalmological and haematological examinations, **only kidneys, stomach, renal and mesenteric lymph nodes examined histopathologically in all animals**; **1.8 mg/kg body weight**: NOAEL; **3.8 mg/kg body weight and above**: relative kidney weights (♂) ↑, absolute kidney weights (♀) ↑, initial signs of chronic progressive nephrosis, low incidence of mild proximal convoluted tubular hyperplasia (♀) ↑, hyperplasia, hyperkeratosis, erosion and ulcers of mild severity of the forestomach of a few animals; **15 mg/kg body weight and above**: ALT ↓ (only an increase is regarded as adverse), urea nitrogen in the serum (♂) ↑, relative kidney weights (♂) ↑, absolute kidney weights (♀) ↑, mild chronic progressive nephrosis, proximal convoluted tubular hyperplasia (♂ after 1 year, ♀ after 29 months), renal tubular adenomas or carcinomas in 4/54 ♂, squamous cell papillomas of the forestomach (irritation), hyperplasia, hyperkeratosis, erosion and ulcers of mild severity of the forestomach, no findings in the glandular part of the stomach; **183 mg/kg body weight**: mortality ↑, body weight gains ↓, relative food intake ↑, dark yellow urine (decreasing intensity after one year, disappeared after 21 ♂ and 23 ♀ months), phosphorus and cholesterol in the serum ↑, albumin in the serum ↓, creatinine in the serum (♂) ↑, urine volume (♂) ↑, specific gravity of urine (♂) ↓, alkaline phosphatase ↓ (only an increase is regarded as adverse), relative liver weights ↑ (no histopathology – in preceding study no histopathological findings in liver), **kidneys**: relative kidney weights ↑, granularity ↑, cortical cysts ↑, pelvic epithelial hyperplasia (♂) ↑, chronic progressive nephrosis, proximal convoluted tubular hyperplasia, tubular adenomas and carcinomas ↑ (Section 5.7),	Fermenta ASC Corporation and SDS Biotech KK 1989

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
		parathyroid: enlargement, hyperplasia is correlated with renal toxicity; **(fore)stomach**: erosion of the glandular stomach but no tumours in this part, squamous cell papillomas and carcinomas, hyperplasia, hyperkeratosis, erosion and ulcers in the squamous epithelium, cysts in the non-glandular stomach → evaluated as a chronic irritant effect of the substance	
rat, CD (SD) BR 50 (70) ♂, 50 (70) ♀	**24 months**, 0, 15, 60, 240, 1200 mg/kg diet (♂: 0, 0.7, 2.7, 10.6, 54 mg/kg body weight and day; ♀: 0, 0.9, 3.3, 13.9, 70 mg/kg body weight and day), purity 99.3%	20 ♂, 20 ♀ examined after 12 months; **0.7/0.9 mg/kg body weight: NOAEL**; **2.7/3.3 mg/kg body weight and above**: dose-dependent hyperplasia and hyperkeratosis of the forestomach ↑, incidence of ulcers of the forestomach ↑, after 12 months incidence of dilated basophilic cortical renal tubules ↑; **10.6/13.9 mg/kg body weight and above**: ALT (♀) ↓ (β-lyase induced depletion of cofactor pyridoxal-5′-phosphate); **54/70 mg/kg body weight**: yellow discoloration of coat, first 4 weeks: body weight gains ↓, ALT (♂) ↓, protein concentration in the urine (♂) ↑, squamous cell adenomas and carcinomas in the forestomach (3/50 ♂; Section 5.7), absolute and relative liver weights (♂) ↑, absolute and relative kidney weights (♂) ↑, progressive glomerulonephrosis (♂) ↑	Vischim Srl 1996
mouse, CD1, 15 ♂, 15 ♀	**13 weeks**, 0, 7.3, 14.3, 48.6, 264, 746 mg/kg diet (♂: 0, 1.2, 2.5, 8.5, 48, 124 mg/kg body weight and day; ♀: 0, 1.4, 3, 10, 51, 141 mg/kg body weight and day), purity 98.4%	only forestomach and kidneys examined histopathologically, no substance-related effects on survival, general condition, body weights, food consumption, all organs unaffected; **2.5/3.0 mg/kg body weight: NOEL for forestomach**; **8.5/10 mg/kg body weight and above**: hyperplasia and hyperkeratosis in the squamous epithelium of the forestomach; **48/51 mg/kg body weight and above**: relative kidney weights (♀) ↑; **124/141 mg/kg body weight**: alkaline phosphatase (♀) ↑, **NOEL for kidneys**	SDS Biotech Corporation 1983 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, CD1, 60 ♂, ♀	**24 months**, 0, 750, 1500, 3000 mg/kg diet (♂: 0, 119, 251, 517 mg/kg body weight and day; ♀: 0, 134, 279, 585 mg/kg body weight and day)	**119/134 mg/kg body weight and above**: hyperplasia and hyperkeratosis in oesophagus and stomach, inflammation and focal necrosis and ulcers in the glandular epithelium of the forestomach, squamous cell carcinomas in the forestomach due to irritant effects, absolute and relative kidney weights dose-dependently ↑, enlargement, discoloration, dilation of pelvis, cysts and nodules, glomerulonephritis, cortical tubular degeneration, cortical cysts, tubular adenomas and carcinomas (♂) not dose-dependent – only significant at lowest dose; **517/585 mg/kg body weight**: slight increase in mortality (♂)	Diamond Shamrock Corporation 1983 b
mouse, CD1, 60 ♂	**24 months**, 0, 10/15, 40, 175, 750 mg/kg diet (0, 1.8, 5.4, 23.2, 99.7 mg/kg body weight and day), purity 98%	lowest dose increased after 18 weeks to 15 mg/kg diet to retain a dose of 1.8 mg/kg body weight and day; **1.8 mg/kg body weight: NOAEL for forestomach**; **5.4 mg/kg body weight and above**: dose-dependent: hyperkeratosis (10/48) in the squamous epithelium of the forestomach (irritant effect), **NOAEL for kidneys**; **23.2 mg/kg body weight and above**: low incidence of hyperplasia in proximally convoluted renal tubules (questionably substance-related), karyomegaly in kidney cells ↑, squamous cell papillomas of the forestomach; **99.7 mg/kg body weight**: relative kidney weights ↑, incidence and severity of proximal convoluted tubular hyperplasia ↑, squamous cell tumours in the forestomach slightly ↑, granular appearance and cortical cysts in kidneys, squamous cell tumours of the forestomach	Fermenta Plant Protection Company 1987 b

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
dog, beagle, 4 ♂, ♀	**13 weeks**, 0, 15, 150, 500 mg/kg body weight and day, gelatine capsule, technical chlorothalonil, purity 97.9%–98.2%	as from day 5 500 mg/kg body weight (as previously at 750 mg/kg body weight: 1 animal died by aspiration of vomit, extreme vomiting in all others); body weight gains, behaviour, ophthalmoscopy and food consumption investigated; weights determined only of brain, liver, kidneys, testes, thyroid; all organs examined histopathologically; no substance-related macroscopic or histopathological changes, including in the kidneys; **15 mg/kg body weight**: ALT ↓ (only an increase is regarded as adverse), **NOAEL**; **150 mg/kg body weight and above**: body weight gains (♂) ↓, cholesterol (♀) ↑, albumin (♂) ↓; **500 mg/kg body weight**: body weight gains (♀) ↓, alkaline phosphatase ↑, blood urea nitrogen (♂) ↓, cholesterol (♂) ↑, albumin (♀) ↓, relative liver weights ↑	ISK Biotech Corporation 1993 a
dog, beagle, 5 ♂, 5 ♀	**12 months**, 0, 15, 150, 500 mg/kg body weight and day, gelatine capsule, technical chlorothalonil, purity 98.3%	**15 mg/kg body weight: NOAEL**; **150 mg/kg body weight and above**: relative liver weights ↑ (♂ 20%; ♀ 26%) without histopathological correlate, dose-dependent increase in brown pigment in cytoplasm of renal tubular epithelium; **500 mg/kg body weight**: body weight gains ↓, total protein (♂) ↓, transient significant increase in cholesterol (♀), serum albumin ↓, relative liver weights ↑ (♂ 22%; ♀ 50%) without histopathological correlate	ISK Biosciences Corporation 1994
dog, no other data	**2 years**, 0, 3, 37.5, ≥ 375 mg/kg body weight and day, no other data	available only as short description; **3 mg/kg body weight: NOAEL**; **37.5 mg/kg body weight: NOAEL for renal toxicity**; **375 mg/kg body weight and above**: glomerulosclerosis and degenerative changes such as renal tubular hypertrophy and dilation, no other data	WHO 2009

ALT: alanine aminotransferase, BrdU: bromodeoxyuridine, NOAEL: no observed adverse effect level, NOEL: no observed effect level, PCNA: proliferating cell nuclear antigen

5.2.3 Dermal application

In the documentation of 1992 there were no data available for the effects after dermal application (documentation "Chlorothalonil" 1993).

After a daily dermal application of chlorothalonil to the dorsal skin of rabbits for 21 days or semiocclusive application to the skin of rats on 5 days per week for 21 days, no systemic effects occurred, although local irritation of the skin was observed (see Table 5; ISK Biosciences Corporation 1996 b; SDS Biotech Corporation 1986).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Chlorothalonil is a mild skin irritant (documentation "Chlorothalonil" 1993; WHO 2009).

In a study carried out in accordance with OECD Test Guideline 404 not described in the documentation of 1992 (documentation "Chlorothalonil" 1993), chlorothalonil moistened with water was slightly irritating to rabbit skin. Very slight erythema was seen in 2 animals after 1 hour and 5 days, respectively, and moderate erythema in one animal after 7 days. Very slight oedema was observed in one animal for 2 days, and in another for three days. In addition, thickening, desquamation and scabbing, and wrinkling of the skin occurred. All symptoms had resolved by day 10 (Zeneca Agrochemicals 2000).

5.3.2 Eyes

Chlorothalonil is severely irritating to corrosive in the rabbit eye (documentation "Chlorothalonil" 1993; WHO 2009).

In a study not described in the documentation of 1992 (documentation "Chlorothalonil" 1993), a dose of 0.1 g technical chlorothalonil of 99.6% purity in powder form was instilled into one eye of 3 male and 3 female New Zealand White rabbits; the eyes were examined after 24, 48 and 72 hours as well as after 7 and 14 days. The untreated eye served as a control. Sodium fluorescein was used to investigate the cornea after 72 hours and 7 and 14 days. At all readings and in all animals, redness of the conjunctiva, chemosis, discharge and blanching were found, small haemorrhages in the conjunctiva were observed up to day 7. Marked signs of irritation were found in all 6 animals, and corneal opacity was seen at all readings up to 14 days. In 2 animals marked chemosis occurred. Fluorescein investigation showed corneal injury in all 6 animals up to 14 days. Pannus and dendritic vascularization were observed in 2 eyes on day 7. On day 14, vascularization of the corneal surface was found in 5 animals. In all animals, detachment of the corneal epithelium and iritis were seen. The highest mean Draize score was 99.9 after 24 hours, the highest individual score was 108, also after 24 hours. Chlorothalonil is severely irritating to the rabbit eye (SDS Biotech Corporation 1982 b).

Table 5 Effects of chlorothalonil after repeated dermal application

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 6 ♂, 6 ♀	**21 days**, daily semiocclusive 6 hours/day, after which the site of application was wiped with a damp cloth, 0, 0.1, 2.5, 50 mg/kg body weight and day in methylcellulose, technical chlorothalonil	application to shaved dorsal skin, about 10% of body surface area, **0 mg/kg body weight**: only vehicle applied; **0.1 mg/kg body weight: local NOAEL**; **2.5 mg/kg body weight and above**: irritation at site of application, mild erythema and oedema from week 1, desquamation in week 3, minimal to mild acanthosis and hyperkeratosis at the end of study, ALT in the serum ↓ (possibly attributable to the impurity pentachlorobenzonitrile (maximum 1%); only an increase is regarded as adverse); **50 mg/kg body weight: systemic NOAEL**, relative kidney weights ↑ (evaluated by the authors as not substance-related due to low weights of controls compared with in 2 further studies); no chlorothalonil and no metabolites in the urine	SDS Biotech Corporation 1986
rat, Fischer 344, 10 ♂	**21 days**, 6 hours/day, 5 days/week, 0, 60, 100, 250, 600 mg/kg body weight and day in 0.2% aqueous methylcellulose, technical chlorothalonil	application to shaved dorsal skin, about 10% of body surface area, animals with collar, substrate with patch attached for 6 hours – after which the substance was washed off, **0 mg/kg body weight**: only vehicle applied; **60 mg/kg body weight and above**: in all exposed animals with similar intensity: mild erythema and desquamation, minimal to moderate hyperkeratosis and squamous epithelial hyperplasia; **250 mg/kg body weight and above**: ALT ↓ (only an increase is regarded as adverse); **600 mg/kg body weight: systemic NOAEL**, hair follicle atrophy	ISK Biosciences Corporation 1996 b

ALT: alanine aminotransferase

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In two earlier experiments using the local lymph node assay (LLNA), groups of 4 animals were treated with 0.02%, 0.1%, 0.2% or 0.5% or with 0.003%, 0.01%, 0.03%, 0.1% or 0.3% chlorothalonil in dimethylformamide. In the first experiment, the stimulation indices were determined to be 11.1, 15.3, 19.2 and 17.2, from which an EC_3 value of 0.002% was extrapolated. In the second experiment, stimulation indices of 2.1, 9.4, 13.8, 18.4 and 27.2 and an EC_3 value of 0.0035% were determined (Boman et al. 2000).

In a modified LLNA which measured the incorporation of bromodeoxyuridine, stimulation indices of 1.0, 1.8, 7.2, and 9.7 were determined with 0.003%, 0.01%, 0.03% and 0.1% chlorothalonil preparations in acetone/olive oil (4:1) (Noda et al. 2006 b).

A positive result was obtained also in the cumulative contact enhancement test: a 3% preparation of chlorothalonil in petrolatum was applied occlusively to the shaved backs of 15 Dunkin Hartley guinea pigs for 24 hours for induction on days 1, 3, 8 and 10. Prior to the third application, they received an intradermal injection of 0.1 ml non-emulsified Freund's complete adjuvant (FCA) on both sides of the occlusive treatment area. The 48-hour occlusive challenge took place 11 days after the final induction with 0.00003% to 0.03% preparations of chlorothalonil in 2-butanone/olive oil (5:6). At the reading after 72 hours, 1 animal reacted to the 0.0001% preparation, 3 animals reacted to the 0.0003% preparation, 9 animals to the 0.001% preparation, 12 animals to the 0.003% preparation and 14 animals each to the 0.01% and 0.03% test preparations. Of the 15 controls, a reaction to the highest test concentration was found only in 1 animal after 72 hours; a reaction to the 0.01% and the 0.03% test preparations was, however, observed in 2 and 3 animals, respectively, after 48 hours. For the concentration that produced a reaction (EC_{50}) in half of the animals at the challenge after induction with 3% chlorothalonil, the authors calculated a value of 0.0031% (for the 48-hour reactions) and 0.00094% (for the 72-hour reactions). A complementary study with groups of 8 to 9 animals is described that were treated for induction occlusively with a 0.03%, 0.1%, 0.3%, 1% or 3% preparation of chlorothalonil in petrolatum. An injection with Freund's adjuvant to avoid non-specific reactions was not used in this study. From the results of this study the authors determined an EC_{50} value of 0.0024% for sensitization after challenge treatment with a 0.03% preparation, based on the 48-hour reactions, or a value of 0.00071% based on the 72-hour reactions. After induction with 0.03% chlorothalonil, 2, 5, 6 and 6 of 8 animals reacted to the challenge with 0.001%, 0.003%, 0.01% and 0.03% chlorothalonil, respectively (Boman et al. 2000).

Also, in a modified maximization test, the results were positive according to Japanese investigations. In this study, groups of 10 Hartley guinea pigs were treated both by intradermal and topical induction with preparations of 0.000005% (group A), 0.00005% (group B), 0.0005% (group C), 0.005% (group D) or 0.05% (group E) chlorothalonil. In all groups pretreated in this way, and in a control group with 5 animals (group F) the challenge was carried out with chlorothalonil preparations in all cited concentrations. In group F, 4 of 5 animals reacted to the highest test concentration, but none of the animals to lower concentrations. In group A, reactions were likewise

found only at the highest test concentration and in all animals after 72 hours and in 9 of 10 animals after 48 hours. In group B, reactions occurred in 2 of 10 animals to 0.0005% chlorothalonil only after 48 hours. Six of 10 animals reacted to 0.005% after 48 hours and 5 of 10 animals after 72 hours. All 10 animals reacted to 0.05% at both time points. In groups C–E all 10 animals reacted to 0.005% and 0.05% chlorothalonil at both readings, whereas 4–8 of 10 animals reacted also to 0.0005% and 1–2 of 10 animals also to 0.00005% chlorothalonil. All 10 animals in which induction with 0.005% chlorothalonil was carried out but none of the 5 controls reacted after challenge also to 0.5% 2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine or pentachloropyridine. After induction with 0.0005% 2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine or with 0.5%/25% (intradermal/topical) pentachloropyridine, 9 and 8 of 10 animals reacted to a challenge with 0.005% chlorothalonil, respectively. Again, no reactions were found in all 5 controls (Noda et al. 2006 a, b).

Summary: More recent investigations confirm the contact sensitization potential of chlorothalonil.

5.4.2 Sensitizing effects on the airways

As before, there are no studies available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

The generation studies with chlorothalonil and 4-hydroxy-2,5,6-trichloroisophthalonitrile are summarized in Table 6.

A 2-generation study in Sprague Dawley rats with chlorothalonil revealed local effects in the forestomach, such as squamous cell hyperplasia and hyperkeratosis, and renal toxicity, such as tubular epithelial hyperplasia and hypertrophy, in animals of the F0 and F1 generations at 45 mg/kg body weight and day and above, in both generations to a similar degree. There were no effects on the litter parameters up to the highest dose of 270 mg/kg body weight and day. A NOAEL for parental toxicity could not be derived. The NOAEL for fertility was 270 mg/kg body weight and day, the highest dose tested (Fermenta ASC Corporation 1990).

The original report of the 3-generation study with chlorothalonil dating from 1967, cited in the documentation of 1992 in very short form, is not available (SDS Biotech Corporation 1967 a, b in documentation "Chlorothalonil" 1993).

In a 1-generation study in Sprague Dawley rats with 4-hydroxy-2,5,6-trichloroisophthalonitrile, reduced body weights occurred in the juvenile animals of the F1 generation at 5.4 mg/kg body weight and day and above. In the F1 generation, the survival index was reduced in the foetuses at 10.8 mg/kg body weight and day, however not on a per litter basis. The NOAEL for parental toxicity was 2.7 mg/kg body weight and day, as a result of reduced body weights at 5.4 mg/kg body weight and day, and the NOAEL for fertility was 10.8 mg/kg body weight and day, the highest dose tested (SDS Biotech Corporation 1982 a).

Table 6 Generation studies with chlorothalonil or 4-hydroxy-2,5,6-trichloroisophthalonitrile

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 35 ♂/35 ♀	**2-generation study**, 0, 500, 1500, 3000 mg chlorothalonil/kg diet (F0 ♂: 0, 23–53 (MV 45), 68–157 (MV 135), 145–292 (MV 270) mg chlorothalonil/kg body weight and day; F0 ♀: 0, 31–50 (MV 45), 94–154 (MV 135), 201–278 (MV 270) mg/kg body weight and day), animals mated 2 times (F0, F1b), PND 4: reduction to 8 offspring/dam, purity: 98.1%	**no NOAEL parental toxicity,** findings in forestomach and kidneys in F0 and F1 with similar severity at all doses (♂) or at middle dose and above (♀); **45 mg/kg body weight**[a)] **and above**: F0: forestomach: thickening of mucosa, squamous cell hyperplasia and hyperkeratosis, kidneys (♂): distension, discoloration, tubular epithelial hyperplasia and hypertrophy, pigmentation of the cortex, F1: forestomach: squamous cell hyperplasia and hyperkeratosis, kidneys (♂): distension, discoloration, tubular epithelial hyperplasia and hypertrophy, foci with clear cell hyperplasia, pigmentation of cortex, karyomegaly; **135 mg/kg body weight and above**: F0/F1: kidneys (♀): distension, discoloration, tubular epithelial hyperplasia and hypertrophy, pigmentation of cortex; F0: body weights (♂) ↓; F1: body weight gains ↓, relative food consumption ↑; **270 mg/kg body weight NOAEL foetotoxicity and fertility;** **270 mg/kg body weight**: F0: kidneys (♂): 1 tubular adenoma and carcinoma; F0: body weights (♀) ↓; F1/F2: body weight at PND 21 compared to control animals ↓ at all 4 matings probably due to start of food uptake; F1: body weights (♀) ↓; no unusual findings: mortality, physical condition, reproduction indices, duration of gestation, survival of offspring	Fermenta ASC Corporation 1990

Table 6 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 12 ♂/24 ♀	**1-generation study**, 0, 10, 20, 30, 60, 120 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg diet (F0 ♂: 0, 0.4–1.0, 0.8–2.1, 1.1–3.0 (MV 2.7), 2.4–6.0 (MV 5.4), 4.9–11.8 (MV 10.8) mg/kg body weight and day; F0 ♀: 0, 0.6–1.1, 1.1–2.2, 1.8–3.3 (MV 2.7), 3.4–6.5 (MV 5.4), 6.6–12.5 (MV 10.8) mg/kg body weight and day; no data for F1), animals mated 2 times, purity: 99%	**2.7 mg/kg body weight**[a]**: NOAEL parental toxicity;** **5.4 mg/kg body weight**: F0 and F1b: body weights ↓ (on average 11.7%; PND 10, 14, 21); **10.8 mg/kg body weight: NOAEL foetotoxicity and fertility;** **10.8 mg/kg body weight**: F0 ♂: body weights ↓ (< 6%, during growth phase week 0–18), F1a and F1b: body weights ↓ (on average by 20%; PND 7, 14, 21); F1: survival index ↓ (only related to foetal basis, related to litter basis not statistically significant); without unusual findings: mortality, physical condition, food intake, mating, incidence of pregnancy, necropsy (conspicuous animals), maternal body weights during gestation	SDS Biotech Corporation 1982 a
rat, Sprague Dawley, 15 ♂/30 ♀	**3-generation study**, 0, 10, 60, 125 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg diet (F0 ♂: 0, 0.5–1.0 (MV 0.9), 2.8–5.9 (MV 5.4), 6.1–12.3 (MV 11.3) mg/kg body weight and day; F1b ♂: 0, 0.4–1.2, 2.8–7.3, 5.4–16.9 mg/kg body weight and day; F2b ♂: 0, 0.4–1.3, 2.7–7.9, 5.2–16.7 mg/kg body weight and day; F0 ♀: 0, 0.6–1.2, 3.9–7.1, 8.0–14.6 mg/kg body weight and day; F1b ♀: 0, 0.6–1.2, 3.9–7.2, 8.0–16.7 mg/kg body weight and day; F2b ♀: 0, 0.6–1.3, 3.8–7.9, 7.8–17.9 mg/kg body weight and day), animals mated 2 times (F0, F1b, F2b), purity: 99%	**0.9 mg/kg body weight**[a]**: NOAEL parental toxicity;** **5.4 mg/kg body weight and above**: F0: body weights ↓ (during growth and adult phase); F1, F2, F3: body weights ↓ (during the parental growth phase and PND 14, 21); **11.3 mg/kg body weight: NOAEL foetotoxicity and fertility;** no unusual findings: mortality, general condition, food intake, duration of gestation, mating, incidence of pregnancy, maternal body weight gains during gestation and lactation, offspring: survival, weights at birth, necropsy (all animals), histological examination (at necropsy conspicuous animals and 5 animals/sex, dose, generation)	Diamond Shamrock Corporation 1981 b

[a] conversion factor 0.09 subchronic according to EFSA (2012);
MV: mean value ♂ and ♀, PND: postnatal day

In a 3-generation study in Sprague Dawley rats with 4-hydroxy-2,5,6-trichloroisophthalonitrile, body weights during the growth and adult phases were reduced in the F0 generation at 5.4 mg/kg body weight and day and above. Reduced body weights were found also in the juvenile F1 to F3 animals. The litter parameters were unaffected up to the high dose of 11.3 mg/kg body weight and day. The NOAEL for parental toxicity was 0.9 mg/kg body weight and day, and the NOAEL for fertility was 11.3 mg/kg body weight and day, the highest dose tested (Diamond Shamrock Corporation 1981 b).

5.5.2 Developmental toxicity

The developmental toxicity studies after oral administration of chlorothalonil or 4-hydroxy-2,5,6-trichloroisophthalonitrile are shown in Table 7.

Rat

In a study of prenatal developmental toxicity with chlorothalonil in rats similar to OECD Test Guideline 414 with gavage administration from gestation days 6 to 15, an increase in the number of early resorptions that was not statistically significant and an increase in postimplantation losses occurred at the highest dose of 400 mg/kg body weight and day. The reduction in litter size was due to one dam which was found to have 17 implantation sites with 16 early resorptions. When this dam is not included in the evaluation, an average postimplantation loss of 1.2 is obtained, instead of 2.1, which, however, is still above the values of historical controls. The authors regarded this effect as substance-related. At this dose, however, severe maternal toxicity occurred. In their summary, the authors associated the increased number of early embryonal mortalities with the severe maternal toxicity (SDS Biotech Corporation 1983 b). As, in the view of the Commission, the effects cannot be attributed unequivocally to the toxicity in the dams, the NAEL for developmental and maternal toxicity is considered to be 200 mg/kg body weight and day.

In a prenatal developmental toxicity study, 4-hydroxy-2,5,6-trichloroisophthalonitrile was administered to rats by gavage from gestation days 0 to 19. Developmental toxicity, such as reduced body weights and an increased incidence of a 14th rib, and maternal toxicity, such as delayed body weight gains and reduced food intake, were observed after doses of 15 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg body weight and day and above. The NOAEL for these effects was 5 mg/kg body weight and day (Zeneca 1998).

From a two generation study with chlorothalonil and a one and three-generation study in rats with 4-hydroxy-2,5,6-trichloroisophthalonitrile and dietary administration, NOAELs for foetotoxicity of 270 mg chlorothalonil/kg body weight and day (Fermenta ASC Corporation 1990) and 10.8 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg body weight and day (SDS Biotech Corporation 1982 a) and 11.3 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg body weight and day (Diamond Shamrock Corporation 1981 b), respectively, were obtained, which were the highest doses tested in each case. Maternal toxicity was observed in all three studies at these dose levels (see Table 6).

Table 7 Developmental toxicity studies with oral administration of chlorothalonil or 4-hydroxy-2,5,6-trichloroisophthalonitrile

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 6 ♀	**GD 1–6**, 0, 200 mg chlorothalonil/kg body weight and day, gavage, examined on PND 21	no unusual findings in: dams: body weights, weights of placenta, ovaries, gravid uterus; foetuses: developmental parameters such as pinna detachment, fur development, teeth eruption, opening of vagina, ears, eyes, testicular descent, swimming tests on days 7, 17 and 21 after birth, the study is not used for assessment of developmental toxicity, as it only covered the gestation days up to implantation and not organogenesis	de Castro et al. 2000
rat, Sprague Dawley COBS CD, 25 ♀	**GD 6–15**, 0, 25, 100, 400 mg chlorothalonil/kg body weight and day, gavage, purity: 98%, vehicle: 0.5% methylcellulose (w/v), examination GD 20, Test Guideline for Registration of Pesticides in US Hazard Evaluation, similar to OECD Test Guideline 414, no evaluation on litter basis	**100 mg/kg body weight: NOAEL developmental and maternal toxicity**; **400 mg/kg body weight**: dams: 3 animals died, soft faeces, hair loss in some animals, during exposure: food intake ↓, body weight gains ↓, number of early resorptions ↑ (2.0 ± 3.7; controls: 0.8 ± 0.9) and postimplantation losses ↑ (2.1 ± 3.6; controls: 0.8 ± 0.9; not statistically significant, high variation in post-implantation losses); In the Commission's view: increase in premature embryonal mortalities not definitely attributable to high maternal toxicity; foetuses: no unusual findings: external, visceral, skeletal variations and malformations, body weights	SDS Biotech Corporation 1983 b

Table 7 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley CD, 24 ♀	**GD 0–19**, 0, 5, 15, 25 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg body weight and day, gavage, purity: 100%, vehicle: 1% methylcellulose, examination GD 20, Test Guideline of EPA Health Effects Test Guidelines OPPTS	**5 mg/kg body weight: NOAEL developmental and maternal toxicity**; **15 mg/kg body weight and above**: dams: body weights ↓, body weight gains ↓, food intake ↓, changes in haematological parameters, number of corpora lutea ↓; foetuses: body weights ↓, incidence of 14th rib per litter ↑, postimplantation losses ↑ (not statistically significant); **25 mg/kg body weight**: dams: incidence of red discoloration in anogenital region or red discharge from the vagina ↑; foetuses: number of live foetuses ↓, postimplantation losses per foetus and per litter ↑, number of early or late resorptions per foetus and per litter ↑; NOAEL for maternal toxicity of a similar order of magnitude as that found in generation studies	Zeneca 1998
mouse, ICR (CD-1), 30 ♀	**GD 6–15**, 0, 100, 400, 600 mg chlorothalonil/kg body weight and day, gavage, purity: 97%, vehicle: corn oil, examination GD 18, similar to OECD Test Guideline 414	**100 mg/kg body weight: NOAEL developmental and maternal toxicity**; **400 mg/kg body weight and above**: dams: body weights and body weight gains ↓, weakness, activity ↓; foetuses: body weights ↓, postimplantation losses ↑, number of live foetuses/litter ↓, number of early resorptions/litter ↑, percentage of litters with resorptions ↑; **600 mg/kg body weight**: dams: absolute weights of kidneys and liver ↑, absolute weights of placenta ↓; no unusual findings with regard to: mortality, number of implantations/litter, sex ratio, teratogenicity (external, skeletal, visceral anomalies)	Farag et al. 2006

Table 7 (continued)

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 20 ♀	**GD 7–19**, 0, 5, 10, 20 mg chlorothalonil/kg body weight and day, gavage, purity: 98.1%, vehicle: 0.5% methylcellulose, examination GD 30, OECD Test Guideline 414	**10 mg/kg body weight: NOAEL maternal toxicity;** **20 mg/kg body weight**: dams: food intake ↓, body weights ↓; **20 mg/kg body weight: NOAEL developmental toxicity;** no substance-related findings in the foetuses: no external, visceral or skeletal anomalies or other toxicity findings	Fermenta Plant Protection Company 1988 b
rabbit, Dutch Belted, 10 ♀	**GD 6–18**, 0, 1, 2.5, 5.0 mg 4-hydroxy-2,5,6-trichloroisophthalonitrile/kg body weight and day, gavage, purity: not specified, vehicle: 0.5% methylcellulose, examination on GD 28	**5 mg/kg body weight**: dams: in some animals hypothermia, hypoactivity, number of dams with miscarriages ↑ (4/9, of which 2 died, these were found to have haemorrhages in the lungs, which can be assumed to have resulted from an intubation error); no unusual findings: number of corpora lutea, number of implantations, number of dead or resorbed foetuses, number of live foetuses/group, sex ratio, external, skeletal and visceral anomalies; dams: body weights; study is not used for this evaluation, as the number of animals was too small and the documentation inadequate	Diamond Shamrock Corporation 1976

GD: gestation day, PND: postnatal day

Mouse

In a prenatal developmental toxicity study in ICR(CD-1) mice similar to OECD Test Guideline 414, doses of 400 and 600 mg/kg body weight and day caused increased postimplantation losses, reduced numbers of live foetuses per litter, increased numbers of early resorptions per litter as well as an increase in the percentage of litters with resorptions. At these dose levels, also maternal toxicity was found in the form of reduced body weights, delayed body weight gains and reduced activity. The NOAEL for developmental and maternal toxicity was 100 mg/kg body weight and day (Farag et al. 2006).

Rabbit

In a prenatal developmental toxicity study carried out according to OECD Test Guideline 414, no developmental toxicity occurred in New Zealand White rabbits at the highest dose tested of 20 mg/kg body weight and day. At this dose, reduced food intake and body weight loss were observed in the dams (Fermenta Plant Protection Company 1988 b). The NOAEL for developmental toxicity was therefore 20 mg/kg body weight and day, and the NOAEL for maternal toxicity 10 mg/kg body weight and day.

The original study with Japanese White rabbits cited in the documentation of 1992 is not available. In the dams the body weights were reduced at the highest dose tested of 50 mg/kg body weight; no effects were observed in the offspring (Shirasu and Teramoto 1975 in documentation "Chlorothalonil" 1993).

Summary: In prenatal developmental toxicity studies similar to OECD Test Guideline 414 with administration by gavage, chlorothalonil caused an increase in postimplantation losses in rats and mice at doses of 400 mg chlorothalonil/kg body weight and day administered from gestation days 6 to 15. In rabbits, no developmental toxicity occurred up to the highest dose tested of 20 mg chlorothalonil/kg body weight and day administered from gestation days 7 to 19 (Fermenta Plant Protection Company 1988 b). In all species, maternal toxicity in the form of reduced body weights or delayed body weight gains occurred at these dose levels and above. The N(O)AELs for developmental toxicity were 200, 100 and 20 mg/kg body weight and day in rats, mice and rabbits, respectively.

5.6 Genotoxicity

5.6.1 In vitro

The investigations of the genotoxicity of chlorothalonil described in the documentation of 1992 (documentation "Chlorothalonil" 1993) yielded mostly negative results and are shown together with the studies published subsequently in Table 8.

The Daconil formulation did not produce DNA adducts at a concentration of 1 mM/plate in a study using Aroclor-induced rat liver S9 in an NADPH-generating system and calf thymus DNA (Shah et al. 1997).

Table 8 Genotoxicity of chlorothalonil in vitro

Endpoint	Test system	Substance	Concentration range [µg/plate]*	Effective concentration*	Cytotoxicity*	Results		References
						–m.a	+m.a	
rec-assay	Bacillus subtilis M45(rec⁻), H17(rec⁺)	chlorothalonil, purity 99.3%	2–200			– –	– –	Diamond Shamrock Corporation undated
DNA adducts	NADPH-generating system from Aroclor-activated rat liver S9 and calf thymus DNA	Daconil[d], no other data	1 mM			n.ex	–	Shah et al. 1997
gene mutation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	chlorothalonil, purity 97.8%	1–100			–	–	Diamond Shamrock Corporation 1977 c
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	chlorothalonil, purity not specified	1–1000		2000	–	–[a]	SDS Biotech Corporation 1984 a
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	chlorothalonil, technical purity not specified	TA100: 10–10 000 0.16–16 (–m.a.) 0.5–50 (+m.a.)		33 (–m.a.) 100[b] (+m.a.)	–	–[a]	SDS Biotech Corporation 1984 b
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	chlorothalonil, purity 99.3%	1–10		10 (–m.a.)	–	–	Diamond Shamrock Corporation undated
	Escherichia coli WP2 uvr A	chlorothalonil, purity 99.3%	1–500 2–100			–	–	Diamond Shamrock Corporation undated

Table 8 (continued)

Endpoint	Test system	Substance	Concentration range [µg/plate]*	Effective concentration*	Cytotoxicity*	Results		References
						–m.a	+m.a	
DNA repair test	Salmonella typhimurium TA1538, TA1978	chlorothalonil, purity not specified, in DMSO	2, 10, 20 µl/plate of a 1 mg/ml solution			–	–	Diamond Shamrock Corporation 1977 a
DNA SB, comet assay	CHO cells	chlorothalonil, purity not specified	0.5–40 µmol/l	0.5 µmol/l	2 µmol/l	+	n.ex	Godard et al. 1999
CA	CHO cells (CHO-K1)	chlorothalonil, purity 98.8%	0.03–0.3 µg/ml (–m.a.) 0.3–6 µg/ml (+m.a.)	0.3 µg/ml	1 µg/ml, cell cycle delayed (–m.a) 10 µg/ml (+m.a.)	+[c]	–	Fermenta Plant Protection Company 1988 c
gene mutation ouabain	V79, Balb/3T3 cells	chlorothalonil, purity 97.8%	V79: 0.3 µg/ml Balb/3T3: 0.03, 0.3 µg/ml		V79: 0.3 µg/ml Balb/3T3: 0.03 µg/ml	–	–	Diamond Shamrock Corporation 1977 b

*) if not otherwise specified, figure given relates to µg/plate
[a] metabolic activation by Aroclor-induced kidney microsomes of the F344 rat
[b] determined only for TA100
[c] severely damaged and pulverized cells were included in the count as 10 aberrations (no other data), this is not in accordance with present procedures
[d] Daconil: chlorothalonil formulation, for example 500 g/l formulation
DNA SB: test for DNA strand breaks, CA: chromosomal aberration, n.ex: not examined

In a study published in 1999, CHO (Chinese hamster ovary) cells were treated for one hour with 0, 0.5, 2, 10 and 40 µmol/l and investigated for DNA strand breaks after 2, 6 and 24 hours by means of alkaline comet assay. A dose-dependent increase in cells with DNA strand breaks without any effect on the viability of the cells was found at concentrations of 0.5 µmol/l and above. At concentrations of 2 µmol/l and above, the number of severely damaged cells increased, which correlated with a decrease in cell viability (Godard et al. 1999).

Summary: Chlorothalonil did not induce mutations in Salmonella typhimurium and in mammalian cells in vitro. DNA strand breaks and chromosomal aberrations were induced in CHO cells without metabolic activation at concentrations close to those causing cytotoxicity.

5.6.2 In vivo

In vivo studies of the genotoxicity of chlorothalonil described in the documentation of 1992 (documentation "Chlorothalonil" 1993) yielded negative or unclear results.

In WHO (2009), chlorothalonil was evaluated as not genotoxic in vivo due to the almost exclusively negative results in the studies.

Other studies are available which were not cited in the documentation of 1992 (documentation "Chlorothalonil" 1993) or in WHO (2009). These are described in greater detail below.

Male Sprague Dawley rats were given single oral chlorothalonil doses of 0, 200 or 2000 mg/kg body weight in corn oil. Whole blood, bone marrow, thymus, liver, renal cortex and distal intestine were investigated using the comet assay for DNA strand breaks after 2, 6 or 24 hours. There was no substance-related increase in the incidence of DNA strand breaks in any of the organs (Godard et al. 1999).

Chromosomal aberrations in the bone marrow were likewise not found after single oral doses of technical chlorothalonil in male mice (up to 2500 mg/kg body weight) and male rats (up to 5000 mg/kg body weight) and examination after 6, 24 and 48 hours (SDS Biotech Corporation 1985 a, b).

Chlorothalonil did not produce chromosomal aberrations in the bone marrow in rats after 5-day oral administration (up to 2000 mg/kg body weight, examination after 6 and 24 hours) or in Chinese hamsters (up to 750 mg/kg body weight, examination after 6 and 24 hours) (Mizens et al. 1998). The publication summarized the studies of Kajiwara and Furusho (1994), ISK Biosciences Corporation (1995 b) and SDS Biotech Corporation (1985 d) described in WHO (2009).

The micronucleus test yielded negative results for mice, rats and hamsters after administration of the maximum tolerable oral doses (documentation "Chlorothalonil" 1993).

In a study without meaningful results, mice were positioned in cages at distances of 0, 30 or 100 metres downwind of a potato field, over which chlorothalonil was sprayed. Thereafter, DNA strand breaks in the leukocytes of the mice were determined by comet assay. There was no increase in DNA strand breaks (Garron et al. 2012); however, the exposure levels were not recorded.

The data from the genotoxicity studies in vivo are given in Table 9.

Table 9 Genotoxicity of chlorothalonil in vivo

Test system		Dose	Result	Remarks	References
DNA SB, comet assay, blood, bone marrow, thymus, liver, renal cortex, distal intestine	rat, Sprague Dawley, groups of 3	single, gavage, 0, 200, 2000 mg/kg body weight, purity not specified, 2, 6 and 24 hours	–		Godard et al. 1999
CA, bone marrow	mouse, Swiss, 10 ♂	twice, gavage, 0, 4, 20, 100, 500, 2500 mg/kg body weight and day, purity 98.2%, 24 hours	–	2500 mg/kg body weight: mortality 1/10	SDS Biotech Corporation 1983 c
CA, bone marrow	mouse, Swiss, 10 ♂	single, gavage, 0, 250, 1250, 2500 mg/kg body weight, purity 98.2%, 6, 24 and 48 hours	–	1250 mg/kg body weight and above: mitotic index (MI) ↓; range-finding study: 5000 mg/kg body weight and above: mortality 5/5	SDS Biotech Corporation 1985 a
CA, bone marrow	rat, Wistar, 10 ♂	twice, gavage, 0, 8, 40, 200, 1000, 5000 mg/kg body weight and day, purity 98.2%, 24 hours	–		SDS Biotech Corporation 1983 c
CA, bone marrow	rat, Wistar, 10 ♂	single, gavage, 0, 500, 2500, 5000 mg/kg body weight, purity 98.2%, 6, 24 and 48 hours	–	range-finding study: 10 000 mg/kg body weight: mortality 1/4	SDS Biotech Corporation 1985 a

Table 9 (continued)

Test system		Dose	Result	Remarks	References
CA, bone marrow	rat, F344, 5 ♂	five times, gavage, 0, 500, 1000, 2000 mg/kg body weight and day, purity 98.8%, 6 and 24 hours	–	MTD 2000 mg/kg body weight; no cytotoxicity (MI)	Kajiwara and Furusho 1994 in WHO 2009; Mizens et al. 1998
CA, bone marrow	hamster, Chinese, 10 ♂	single, gavage, 0, 500, 2500, 5000 mg/kg body weight, purity 98.2%, 6, 24 and 48 hours	–	1250 mg/kg body weight and above: mitotic index ↓; range-finding study: 5000 mg/kg body weight and above: mortality 1/5	Siou 1985 b in WHO 2009
CA, bone marrow	hamster, Chinese, 10 ♂	twice, gavage, 0, 8, 40, 200, 1000, 5000 mg/kg body weight and day, purity 98.2%, 24 hours	–	200 mg/kg body weight and above: mortality; 5000 mg/kg body weight: toxicity, mortality 4/13; range-finding study: 10 000 mg/kg body weight: mortality 5/5	SDS Biotech Corporation 1983 c
CA, bone marrow	hamster, Chinese, 10 ♂	five times, gavage, 0, 50, 125, 250 mg/kg body weight and day purity 98.2%	+/–	50 and 250 mg/kg body weight CA 3–4-fold not significantly ↑, no DRR; 50 mg/kg body weight and above: mortality 1/5; MI ↑	Siou 1985 a in WHO 2009; Mizens et al. 1998
CA, bone marrow	hamster, Chinese, 10 ♂	five times, gavage, 0, 187.5, 375, 750 mg/kg body weight and day purity 98.3% (technical); 6 and 24 hours	–	750 mg/kg body weight: body weights ↓ by 12%; mortality 4/10: → MTD	Mizens and Laveglia 1995 in WHO 2009; Mizens et al. 1998

Table 9 (continued)

Test system		Dose	Result	Remarks	References
MN, bone marrow	mouse, Swiss, 7 ♂, 7 ♀	single, gavage, ♂: 0, 1000, 5000, 10 000 mg/kg body weight, ♀: 0, 500, 2500, 5000 mg/kg body weight, 24, 48 and 72 hours	+ only at 10 000 mg/kg body weight at 48-hour reading –	♂: 10 000 mg/kg body weight: PCE/NCE ratio ↓ (48 and 72 hours), marked toxicity; ♀: 5000 mg/kg body weight: PCE/NCE ratio ↓ (48 hours)	Siou and Lerond-Conan 1985 in WHO 2009
MN, bone marrow	mouse, Swiss, 10 ♂	twice, gavage, 0, 4, 20, 100, 500, 2500 mg/kg body weight and day, purity 98.2%, 24 hours	–	5000 mg/kg body weight: mortality 5/5	SDS Biotech Corporation 1983 d
MN, bone marrow	rat, Wistar, 10 ♂	twice, gavage, 0, 8, 40, 200, 1000, 5000 mg/kg body weight and day, purity 98.2%, 24 hours	–	5000 mg/kg body weight: MTD	SDS Biotech Corporation 1983 d
MN, bone marrow	hamster, Chinese, 10 ♂	twice, gavage, 0, 4, 20.100, 500, 2500 mg/kg body weight and day, purity 98.2%, 24 hours	–	5000 mg/kg body weight: mortality 5/5	SDS Biotech Corporation 1983 d

CA: test for structural chromosomal aberrations; DNA SB: test for DNA strand breaks; DRR: dose-response relationship; MN: micronucleus test; MTD: maximum tolerable dose; PCE/NCE: polychromatic erythrocytes/normochromatic erythrocytes

Summary: Chlorothalonil did not induce mutations in Salmonella typhimurium and in mammalian cells in vitro. In CHO cells, DNA strand breaks were found in the comet assay, and in a test for chromosomal aberrations without the addition of a metabolic activation system chromosomal abeations were induced at a concentration close to that causing cytotoxicity. In vivo, the results of tests for DNA strand breaks in different tissues, for chromosomal aberrations in the bone marrow of mice and rats and for micronuclei in rats and hamsters were negative. The two unclear or positive results in a chromosomal aberration test with hamsters and a micronucleus test with mice were accompanied by cytotoxicity, mortality and marked toxicity, and can therefore be regarded as indicating a secondary genotoxic effect. Overall, chlorothalonil is regarded as not genotoxic.

5.7 Carcinogenicity

As described in the documentation of 1992 (documentation "Chlorothalonil" 1993), after long-term oral administration of chlorothalonil, papillomas and carcinomas of the squamous epithelium occurred in the forestomach of some animals (rats, mice, both sexes) and, particularly in Fischer 344 rats, carcinomas of the renal proximal tubular epithelium were induced, although not in Osborne-Mendel and Sprague Dawley rats.

In a 2-year feeding study with dogs, no tumours were found (no other data; WHO 2009).

Carcinogenicity studies in rats not yet described in the documentation of 1992 (documentation "Chlorothalonil" 1993) are listed in Table 10 and confirm doses without carcinogenic effects: 10 mg/kg body weight and day in Sprague Dawley rats (Vischim Srl 1996) and 3.8 mg/kg in Fischer 344 rats (Fermenta ASC Corporation and SDS Biotech KK 1989).

The formation of the tumours in the forestomach of rats and mice is presumably due to the irritant effect of the substance, which is more intense in the non-glandular forestomach of rodents than in the human stomach, which has glands. The renal tumours are probably attributable to metabolically formed glutathione conjugates, which are converted to cytotoxic thiol compounds specifically in the kidneys of rodents, also with the participation of β-lyase. The presence of such thiol compounds was found in vivo. Evidence that the thiol compounds are exclusively responsible for the renal toxicity of chlorothalonil has not been provided to date, although this has been confirmed for structurally similar substances.

The mechanism is assumed not to be genotoxic and could occur in humans (WHO 2009).

Table 10 Studies of the carcinogenicity of chlorothalonil

Author:	Vischim Srl 1996					
Substance:	chlorothalonil (purity 99.3%)					
Species:	**rat**, CD (SD) BR, 70 ♂, 70 ♀					
Administration route:	with the diet					
Doses:	0, 15, 60, 240, 1200 mg/kg diet (♂: 0, 0.7, 2.7, 10.6, 54 mg/kg body weight and day; ♀: 0, 0.9, 3.3, 13.9, 70 mg/kg body weight and day)					
Duration:	2 years, daily					
Toxicity:	at 10.6/13.9 mg/kg body weight and above: ALT activity ♀ ↓					
		Dose (mg/kg body weight and day)				
		0	**0.7/0.9**	**2.7/3.3**	**10.6/13.9**	**54/70**
Survivors	♂	19/50 (38%)	16/50 (32%)	15/50 (30%)	14/50 (28%)	19/50 (38%)
	♀	17/50 (34%)	20/50 (40%)	13/50 (26%)	15/50 (30%)	16/50 (32%)
Liver						
hepatocellular adenomas terminal incidence	♂	1/50 (2%)	0/50 (0%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
	♀	1/50 (2%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
hepatocellular carcinomas terminal incidence	♂	2/50 (4%)	1/50 (2%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Forestomach						
squamous papillomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
squamous carcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Kidneys						
adenomas	♂	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
carcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
	♀	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Pituitary						
adenomas	♂	12/50 (24%)	16/41 (39%)	16/40 (40%)	19/43* (44%)	16/50 (32%)
	♀	28/50 (56%)	33/50 (66%)	37/50* (74%)	36/50 (72%)	30/50 (60%)
adenocarcinomas	♂	0/50 (0%)	0/41 (0%)	0/40 (0%)	0/43 (0%)	0/50 (0%)
	♀	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/50 (2%)	0/50 (0%)

Table 10 (continued)

Author:	Fermenta ASC Corporation and SDS Biotech KK 1989					
Substance:	technical chlorothalonil (purity 98.3%)					
Species:	**rat**, Fischer 344, 65 ♂, 65 ♀					
Administration route:	with the diet					
Doses:	0, 1.8, 3.8, 15, 183 mg/kg body weight and day, **only kidneys, stomach, renal and mesenteric lymph nodes examined histopathologically in all animals**					
Duration:	♂ 23–26 months, ♀ 29 months, daily					
Toxicity:	3.8 mg/kg body weight and above: dose-dependent renal toxicity					
		Dose (mg/kg body weight and day)				
		0	**1.8**	**3.8**	**15**	**183**
Survivors	♂	32/55	33/55	33/55	21/55	11/55
	♀	25/55	29/55	19/55	20/55	10/55
Renal tubules						
adenomas	♂	1/55 (2%)	1/54 (2%)	1/54 (2%)	3/54 (6%)	17/55** (31%)
	♀	0/55 (0%)	0/54 (0%)	0/55 (0%)	0/53 (0%)	24/55** (44%)
carcinomas	♂	0/55 (0%)	0/54 (0%)	0/54 (0%)	1/54 (2%)	7/55* (13%)
	♀	0/55 (0%)	0/54 (0%)	0/55 (0%)	0/53 (0%)	11/55** (20%)
clear cell hyperplasia	♂	1/55 (2%)	3/54 (6%)	1/54 (2%)	3/54 (6%)	10/55** (18%)
	♀	0/55 (0%)	0/54 (0%)	1/55 (2%)	6/53* (11%)	29/55** (53%)
Forestomach						
papillomas	♂	0/55 (0%)	0/54 (0%)	3/54 (6%)	2/54 (4%)	5/55* (9%)
	♀	1/55 (2%)	1/54 (2%)	2/55 (4%)	4/53 (7%)	7/55* (13%)
carcinomas	♂	0/55 (0%)	0/54 (0%)	0/54 (0%)	0/54 (0%)	0/55 (0%)
	♀	1/55 (2%)	0/54 (0%)	0/55 (0%)	1/53 (2%)	3/55 (6%)
hyperplasia[a)]	♂	9/55 (16%)	7/54 (13%)	23/54** (43%)	49/54** (91%)	49/55** (89%)
	♀	6/55 (11%)	7/54 (13%)	24/55** (44%)	42/53** (79%)	49/55** (89%)
hyperkeratosis[a)]	♂	8/55 (15%)	7/54 (13%)	22/54** (41%)	50/54** (93%)	53/55** (96%)
	♀	5/55 (9%)	5/54 (9%)	16/55** (29%)	37/53** (70%)	49/55** (89%)

[a)] hyperplasia and hyperkeratosis increase in incidence and severity with increasing dose
*: $p < 0.05$; **: $p < 0.01$
ALT: alanine aminotransferase

Summary: The increased incidence of forestomach carcinomas in rats is not used for the assessment of carcinogenicity. Due to the particular anatomy of rats and mice, in which there is no glandular epithelium in the forestomach, the strong irritant effects of chlorothalonil cannot be buffered by the formation of a secretion. Consequently, in rodents, the substance acts directly on the epithelium.

In two strains of rat, no increase in the incidence of renal tubular carcinomas was observed (Sprague Dawley rats, at doses of up to 750 mg/kg body weight and day, and Osborne-Mendel rats, at doses of up to 506 mg/kg body weight and day). Only in Fischer 344 rats were the incidences of these tumours increased: increase not significant at 40 mg/kg body weight and day (in 6 of 120 animals), but statistically significant (in 18 of 110 or 120 animals) at 175 mg/kg body weight and day and above. With regard to renal tubular carcinomas, Fischer 344 rats are markedly more sensitive than Sprague Dawley rats. Why there are such large differences in the sensitivity of individual rat strains is unclear.

6 Manifesto (MAK value/classification)

Chlorothalonil is highly irritating in the eye of rabbits; longer-term inhalation studies are not available. After ingestion, renal toxicity was found in rats, mice and dogs.

MAK value. Chlorothalonil was found to be highly irritating in a study of its effects on the rabbit eye and, in a study of acute inhalation toxicity, also in the respiratory tract. However, there are no inhalation studies with repeated exposure. The mechanism of the irritant effect caused by chlorothalonil is not known. It is presumably based on a reaction with proteins containing thiols by substitution of one or several chlorine atoms on the aromatic ring, which are activated by both cyano groups and the other chlorine atoms. From long-term oral toxicity studies with dietary administration of chlorothalonil, a systemic NOAEL of 1.8 mg/kg body weight and day for rats and 5.4 mg/kg body weight and day for mice is obtained. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs to a concentration in workplace air: the daily exposure of the animals compared with the 5 days per week exposure at the workplace (7:5), the species-specific correction values for the rat and the mouse (1:4 and 1:7), the experimental oral absorption (32% in rats, also assumed for mice), the body weight (70 kg) and the respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentrations in air calculated from this are 1.4 (rats) and 2.4 mg chlorothalonil/m^3 (mice). Rats and mice appear to be much more sensitive than humans for the probable β-lyase mediated mechanism by which chlorothalonil causes renal toxicity. Using the standard procedure of the Commission for the extrapolation of the data from the experimental animal study to humans (1:2) and the preferred value approach, a threshold limit value of 0.5 mg/m^3 would be obtained. However, irritation of the airways has been reported by exposed persons in this concentration range (Huang et al. 1995). It is therefore not clear whether this threshold limit value calculated from oral studies would also protect against local irritation. Therefore, a MAK value has not been established for chlorothalonil, and the substance is assigned to Section II b of the List of MAK and BAT Values. Peak limitation is thus not applicable.

Carcinogenicity. On the basis of all available data for genotoxicity and for the mechanism of action, the Commission regards chlorothalonil as not primarily genotoxic.

There are no inhalation studies available. A significant increase in the incidence of renal tubular carcinomas occurred only at the highest dose tested in two oral carcinogenicity studies with Fischer 344 rats. This was not observed, however, in five other carcinogenicity studies with Sprague Dawley rats or one carcinogenicity study with Osborne-Mendel rats, although in some cases markedly higher doses were used than with the Fischer 344 rats. Nevertheless, renal toxicity was found at similar concentrations in both Sprague Dawley and Fischer 344 rats. It is not clear why there are such great differences in reaction in the kidneys.

The kidney tumours are probably induced by metabolically formed thiol compounds, which inhibit mitochondrial breathing and cell proliferation, followed by the induction of hyperplasia and necrosis in the kidneys. Because of the pronounced irritant effect of chlorothalonil, humans are certainly not exposed on a long-term basis to any concentration in the air which could result in renal toxicity. Chlorothalonil has therefore been removed from Category 3B for carcinogens.

Germ cell mutagenicity. There are no investigations available for the effects of the substance in germ cells. In vitro, chlorothalonil did not induce mutations in Salmonella typhimurium and in mammalian cells. In CHO cells, DNA strand breaks and, without metabolic activation, chromosomal aberrations are induced at exposure levels close to the cytotoxic concentration. In vivo, tests for DNA strand breaks in different tissues, for chromosomal aberrations in the bone marrow of mice and rats and for micronuclei in rats and hamsters yielded negative results. Overall, chlorothalonil is regarded as not primarily genotoxic. The substance is therefore not classified in one of the categories for germ cell mutagens.

Prenatal toxicity. In prenatal developmental toxicity studies with gavage administration, chlorothalonil led to an increase in postimplantation losses with simultaneous maternal toxicity in rats and mice after doses of 400 mg/kg body weight and day, given from gestation days 6 to 15. There were no effects on development in rabbits given the highest dose tested of 20 mg chlorothalonil/kg body weight and day from gestation days 7 to 19. The NAEL for developmental toxicity in rats was 200 mg chlorothalonil/kg body weight and day and the NOAELs in mice and rabbits were 100 and 20 mg/kg body weight and day, respectively. In a two-generation study in rats with dietary administration, the NOAELs for foetotoxicity was 270 mg chlorothalonil/kg body weight and day, the highest dose tested. Maternal toxicity also occurred at this level. As no MAK value has been derived, the substance has not been assigned to a Pregnancy Risk Group.

Absorption through the skin. Data from in vitro and in vivo studies are available for the absorption of chlorothalonil through the skin. Using the highest flux determined in vivo with rats, and taking into consideration the much better barrier properties of human skin, compared with rat skin, as clearly demonstrated in vitro, a dermal absorption of 0.99 mg chlorothalonil would be obtained for the exposure of a 2000 cm^2 surface area of skin to an undiluted suspension concentrate for one hour. To assess the amount thus absorbed, a NOAEL of 1.8 mg/kg body weight and day can

be used, which was determined in a long-term feeding study (≥ 23 months) with rats. From the given value, taking into consideration the oral absorption in the rat (32%), the species-specific correction value (1:4), the different exposure frequency at the workplace (7:5), the origin of the data from an animal study (1:2) and a body weight of 70 kg for the person, a systemically tolerable amount of 7.06 mg can be derived. The amount absorbed through the skin is therefore less than 25% of the systemically tolerable amount. Chlorothalonil is therefore not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Since the supplement of 2000 (supplement "Chlorothalonil" 2000, available in German only), only very few more recent clinical findings on the contact-sensitizing effects of chlorothalonil have been published. All of the findings from experimental animal studies indicate, however, that the substance has marked contact-sensitizing potential, so that the designation of chlorothalonil with "Sh" (for substances which cause sensitization of the skin) remains justified. Data for a possible sensitizing effect on the airways were found only in two case descriptions which, however, do not provide sufficient evidence for such an effect. The substance is therefore not designated with "Sa" (for substances which cause sensitization of the airways).

7 References

Aprea C, Centi L, Lunghini L, Banchi B, Forti MA, Sciarra G (2002) Evaluation of respiratory and cutaneous doses of chlorothalonil during re-entry in greenhouses. J Chromatogr B 778: 131–145

Boman A, Montelius J, Rissanen RL, Lidén C (2000) Sensitizing potential of chlorothalonil in the guinea pig and the mouse. Contact Dermatitis 43: 273–279

Chem Service (2014) Safety Data Sheet Chlorothalonil, SDS US (USA Safety Data Sheet), 12. September 2014, http://cdn.chemservice.com/product/msdsnew/External/English/N-11454%20English%20SDS%20US.pdf

De Castro VL, Chiorato SH, Pinto NF (2000) Biological monitoring of embrio-fetal exposure to methamidophos or chorothalonil on rat development. Vet Hum Toxicol 6: 361–365

Diamond Shamrock Corporation (undated) Mutagenicity testing in microbial system. Institute of Environmental Toxicology, CTL/C3289, 0531, Diamond Shamrock Corporation, Cleveland, OH, USA, unpublished report

Diamond Shamrock Corporation (1976) Teratology study in rabbits. International Research and Development Corporation, Datum: 25.02.1976, Diamond Shamrock Corporation, Cleveland, OH, USA, unpublished report

Diamond Shamrock Corporation (1977 a) Activity in a test for differential inhibition of repair deficient and repair competent strains of Salmonella typhimurium: repair test. Microbiological Associates, CTL/C/3382, 0542, June 29, 1977, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1977 b) Activity in an in vitro mammalian cell point mutation assay. Microbiological Associates, CTL/C/3287, 0595, July 22, 1977, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1977 c) Activity in the Salmonella/microsomal assay for bacterial mutagenicity. Microbiological Associates, Doc No. 000-57X-77-0098-001, 0774, June 29, 1977, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1981 a) A 90-day toxicity study of technical chlorothalonil in rats. Diamond Shamrock Corporation, Doc No. 099-5TX-80-0200-006, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1981 b) A three generation reproduction study in rat. Bio/dynamic Inc, NJ, USA, Doc No. 107-5TX-78-0023-002, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1983 a) A 13-week subchronic toxicity study in rats – followed by a 13-week withdrawal period. Huntington Research Centre, Doc No. 562-5TX-81-0213-003, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Diamond Shamrock Corporation (1983 b) A chronic dietary study in mice with technical chlorothalonil. Diamond Shamrock Corporation, Doc No. 108-5TX-79-0102-004, Diamond Shamrock Corporation, Painesville, OH, USA, unpublished report

Draper A, Cullinan P, Campbell C, Jones M, Newman Taylor A (2003) Occupational asthma from fungicides fluazinam and chlorothalonil. Occup Environ Med 60: 76–77

ECB (European Chemicals Bureau) (2000) Chlorothalonil CAS No. 1897-45-6. IUCLID dataset, 18.02.2000, ECB, Ispra, Italy

EFSA (European Food Safety Authority) (2012) Scientific opinion: Guidance on selected default values to be used by the EFSA scientific Committee, scientific panels and units in the absence of actual measured data. EFSA J 10: 2579, http://www.efsa.europa.eu/de/efsajournal/doc/2579.pdf

Enoch SJ, Roberts DW, Cronin MT (2009) Electrophilic reaction chemistry of low molecular weight respiratory sensitizers. Chem Res Toxicol 22: 1447–1453

Farag AT, Karkour TA, El Okazy A (2006) Embryotoxicity of oral administered chlorothalonil in mice. Birth Defects Res B Dev Reprod Toxicol 77: 104–109

Fermenta Plant Protection Company (1987 a) A 90 day study in rats with the mono-glutathione conjugate of chlorothalonil. International Research and Development Corporation, Doc No. 1108-85-0078-TX-006, Fermenta Plant Protection Company, Painesville, OH, USA, unpublished report

Fermenta Plant Protection Company (1987 b) A tumorigenicity study of technical chlorothalonil in male mice. International Research and Development Corporation Inc., Doc No. 1099-84-0077-TX-006, Fermenta Plant Protection Company, Painesville, OH, USA, unpublished report

Fermenta Plant Protection Company (1988 a) A 4-day study in rats with technical chlorothalonil. C.E.R.T.I., Doc No. 1095-86-0091-TX-002, Fermenta Plant Protection Company, Mentor, OH, USA, unpublished report

Fermenta Plant Protection Company (1988 b) A teratology study in rabbits with technical chlorothalonil. Bio/dynamics Inc., Doc No. 1544-87-0060-TX-002, Fermenta Plant Protection Company, Mentor, OH, USA, unpublished report

Fermenta Plant Protection Company (1988 c) In vitro chromosomal aberration assay in Chinese hamster ovary (CHO) cells with technical chlorothalonil. Microbiological Associates Inc., Doc No. 1109-85-0082-TX-002-001, Fermenta Plant Protection Company, Mentor, OH, USA, unpublished report

Fermenta ASC Corporation (1990) A two generation reproduction study in rats with technical chlorothalonil. Department of toxicology and animal metabolism, Doc No. 1722-87-0121-TX-003, Fermenta ASC Corporation, Mentor, OH, USA, unpublished report

Fermenta ASC Corporation, SDS Biotech KK (1989) A tumorigenicity study of technical chlorothalonil in rats. International Research and Development Corporation Inc., Doc No. 1102-84-0103-TX-007, Fermenta ASC Corporation, Mentor, OH, USA and SDS Biotech KK, Tokyo, Japan, unpublished report

Garron C, Knopper LD, Ernst WR, Mineau P (2012) Assessing the genotoxic potential of chlorothalonil drift from potato fields in Prince Edward Island, Canada. Arch Environ Contam Toxicol 62: 222–232

Godard T, Fessard V, Huet S, Mourot A, Deslandes E, Pottier D, Hyrien O, Sichel F, Gauduchon P, Poul J (1999) Comparative in vitro and in vivo assessment of genotoxic effects of etoposide and chlorothalonil by the comet assay. Mutat Res 444: 103–116

Hillenweck A, Corpet DE, Killeen JC Jr, Bliss M Jr, Cravedi JP (1998) Ex vivo gastrointestinal biotransformation of chlorothalonil in the germ-free and conventional rat. Xenobiotica 28: 1017–1028

Hillenweck A, Corpet DE, Killeen JC, Bliss M Jr, Cravedi J-P (1999) Urinary and biliary metabolic patterns of chlorothalonil in germ-free and conventional rats. J Agric Food Chem 47: 2898–2903

Huang J, Aoyama K, Ueda A, Matsushita T (1995) Respiratory effects and skin allergy in workers exposed to tetrachloroisophthalonitrile. Bull Environ Contam Toxicol 55: 320–324

ISK Biotech Corporation (1993 a) A 90-day oral toxicity study in dogs with chlorothalonil. Bio/dynamics, Inc., Doc No. 5210-92-0103-TX-003, ISK Biotech Corporation, Mentor, OH, USA, unpublished report

ISK Biotech Corporation (1993 b) Electron microscopic evaluation of kidneys in male Fischer 344 rats following the oral administration (technical chlorothalonil). Ricerca Inc., Department of toxicology and animal metabolism, Doc No. 1664-87-0089-TX-002, ISK Biotech Corporation, Mentor, OH, USA, unpublished report

ISK Biotech Corporation (1993 c) Acute (four hour) inhalation toxicity (LC50) study in rats with hammer milled technical chlorothalonil. Huntington Research Centre Ltd., 5290-92-0160-TX-002, ISK Biotech Corporation, Mentor, OH, USA, unpublished report

ISK Biosciences Corporation (1994) A chronic (12-month) oral toxicity study in dogs with technical chlorothalonil. Pharmaco LSR Inc., Doc No. 5211-92-0457-TX-003, ISK Biotech Corporation, Mentor, OH, USA, unpublished report

ISK Biosciences Corporation (1995 a) Study to determine the extent and nature of biliary excretion of chlorothalonil and/or metabolites in the dog, part I. Ricerca Inc., Department of toxicology and animal metabolism, Doc No. 5521-93-0319-AM-001, ISK Biosciences Corporation, Mentor, OH, USA, unpublished report

ISK Biosciences Corporation (1995 b) In vivo bone marrow chromosomal analysis in Chinese hamster following multiple dose administration of technical chlorothalonil. Huntingdon Research Center, Doc No. 6005-94-0047-TX-003, ISK Biosciences Corporation, Mentor, OH, USA, unpublished report

ISK Biosciences Corporation (1996 a) A 90-day pilot study for the evaluation of cell proliferation in the kidneys of male rats following the oral administration of technical chlorothalonil. Ricerca Inc., Department of toxicology and animal metabolism, Doc No. 6704-96-0010-TX-003, ISK Biosciences Corporation, Mentor, OH, USA, unpublished report

ISK Biosciences Corporation (1996 b) A 21-day repeated dose dermal toxicity study in rats with technical chlorothalonil. Ricerca Inc., Doc No. 6859-96-0113-TX-002, ISK Biosciences Corporation, Mentor, OH, USA, unpublished report

Kim Y-M, Park K, Jung S-H, Choi J-H, Kim W-C, Joo G-J, Rhee I-K (2004) Chlorothalonil-biotransformation by glutathione S-transferase of Escherichia coli. J Microbiol 42: 42–46

Lensen G, Jungbauer F, Gonçalo M, Coenraads PJ (2007) Airborne irritant contact dermatitis and conjunctivitis after occupational exposure to chlorothalonil in textiles. Contact Dermatitis 57: 181–186

Lensen G, Coenraads PJ, Jungbauer F, Schuttelaar ML (2011) Contact dermatitis caused by chlorothalonil on imported roses: irritant or allergic reaction? Contact Dermatitis 65: 50–51

Matsushita S, Kanekura T, Saruwatari K, Kanzaki T (1996) Photoallergic contact dermatitis due to Daconil. Contact Dermatitis 35: 115–116

Mizens M, Killeen JC Jr, Eilrich GL (1998) The mutagenic potential of chlorothalonil: in vivo chromosome aberration studies. Mutat Res 403: 269–274

Mozzachio AM, Rusiecki JA, Hoppin JA, Mahajan R, Patel R, Beane-Freeman L, Alavanja MCR (2008) Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study. Environ Res 108: 400–403

Müller F, Ackermann P, Margot P (2011) Fungicides, agricultural 2, Individual fungicides, in: Ullmann's encyclopedia of industrial chemistry, Wiley-VCH, Weinheim, https://doi.org/10.1002/14356007.o12o06

Natsch A, Haupt T, Laue H (2011) Relating skin sensitizing potency to chemical reactivity: reactive Michael acceptors inhibit NF-κB signaling and are less sensitizing than SNAr- and SN2-reactive chemicals. Chem Res Toxicol 24: 2018–2027

Noda T, Yamano T, Ooshima T, Shimizu M (2006 a) Contact allergenicity and cross reactivity of 2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine in guinea pig maximization test (Japanese). Seikatsu Eisei 50: 76–83

Noda T, Yamano T, Ooshima T, Shimizu M (2006 b) Contact allergenicity and cross reactivity of 2,4,5,6-tetrachloroisophthalonitrile in a modified guinea pig maximization test (Japanese). Seikatsu Eisei 50: 197–205

Penagos H, Ruepert C, Partanen T, Wesseling C (2004) Pesticide patch test series for the assessment of allergic contact dermatitis among banana plantation workers in Panama. Dermatitis 15: 137–145

Roberts DW, Api AM, Safford RJ, Lalko JF (2015) Principles for identification of high potency category chemicals for which the dermal sensitisation threshold (DST) approach should not be applied. Regul Toxicol Pharmacol 72: 683–693

Rosner E, Klos C, Dekant W (1996) Biotransformation of the fungicide chlorothalonil by glutathione conjugation. Fundam Appl Toxicol 33: 229–234

SDS Biotech Corporation (1982 a) A one-generation reproduction study in rats. Diamond Shamrock Corporation, Doc No. 529-5TX-81-0193-002, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1982 b) Primary eye irritation study in albino rabbits with technical chlorothalonil. Diamond Shamrock Corporation, Doc No. 104-5TX-80-0037-002, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1983 a) A 90-day feeding study in mice with technical chlorothalonil. SDS Biotech Corporation, Doc No. 618-5TX-83-0007-004-DS-2787, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1983 b) A teratology study in rats with technical chlorothalonil. WIL Laboratories Inc, Doc No. 517-5TX-82-0011-003, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1983 c) The chromosomal aberration test in the rat, mouse and hamster using chlorothalonil. C.E.R.T.I., Doc No. 000-5TX-81-0025-002, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1983 d) The micronucleus test in the rat, mouse and hamster using chlorothalonil. C.E.R.T.I., Doc No. 000-5TX-81-0024-004, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1984 a) Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with and without renal activation. Microbiological Associates Inc., Doc No. 694-5TX-84-0093-001, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1984 b) Salmonella/mammalian-microsome plate incorporation assay (Ames test) with and without renal activation with technical chlorothalonil. Microbiological Associates Inc., Doc No. 694-5TX-84-0064-001, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1985 a) In vivo bone marrow chromosomal aberration assay in mice with a single dose of technical chlorothalonil. Department of safety assessment, Doc No. 625-5TX-83-0029-002, SDS-2787, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1985 b) In vivo bone marrow chromosomal aberration assay in rats with a single dose of technical chlorothalonil. Department of safety assessment, Doc No. 625-5TX-83-0028-002, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1985 c) A tumorigenicity study of technical chlorothalonil in rats. SDS Biotech Corporation, Doc No. 099-5TX-80-0234-008, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1985 d) The micronucleus test in mice. C.E.R.T.I., 5TX-84-0071, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech Corporation (1986) 21-day repeated dose dermal toxicity study in albino rabbits with technical chlorothalonil. SDS Biotech Corporation, Doc No. 754-5TX-85-0023-007, SDS Biotech Corporation, Painesville, OH, USA, unpublished report

SDS Biotech KK (1996) Analysis of hyperplastic changes in the stomach and kidney of male rats after 28-day induction by chlorothalonil technical. Center for Safety Assessment of Food, Agricultural Chemicals and Medical Drugs, Report No. 3561 (1996), Test No. 2913 (063-002), September 25, 1996, SDS Biotech KK, Tokyo, Japan, unpublished

Shah RG, Lagueux J, Kapur S, Levallois P, Ayotte P, Tremblay M, Zee J, Poirier GG (1997) Determination of genotoxicity of the metabolites of the pesticides Guthion, Sencor, Lorox, Reglone, Daconil and Admire by 32P-postlabeling. Mol Cell Biochem 169: 177–184

Spencer JR, Bissell SR, Sanborn JR, Schneider FA, Margetich SS, Krieger RI (1991) Chlorothalonil exposure of workers on mechanical tomato harvesters. Toxicol Lett 55: 99–107

Suzuki T, Nojiri H, Isono H, Ochi T (2004) Oxidative damages in isolated rat hepatocytes treated with the organochlorine fungicides captan, dichlofluanid and chlorothalonil. Toxicology 204: 97–109

Syngenta Crop Protection Inc (2001) Chlorothalonil: investigation of renal toxicity in the male Fischer 344 rat following a single oral dose of chlorothalonil. Syngenta Central Toxicology Laboratory, Doc No. CTL/XR6655/Gen/Rept, Syngenta Crop Protection Inc., Greensboro, North Carolina, USA, unpublished report

Syngenta Crop Protection Inc (2005 a) Chlorothalonil: preliminary acute reference dose study in the Fischer 344 rat. Syngenta Central Toxicology Laboratory, AR7493, Syngenta Crop Protection Inc., Greensboro, North Carolina, USA, unpublished report

Syngenta Crop Protection Inc (2005 b) Chlorothalonil: acute reference dose study in the Fischer 344 rat. Syngenta Central Toxicology Laboratory, AR7494, Syngenta Crop Protection Inc., Greensboro, North Carolina, USA, unpublished report

Vischim Srl (1996) Chlorothalonil – potential tumorigenic effects in prolonged dietary administration to rats. Huntingdon Life Sciences Ltd., Doc No. CCM 15/943286, Vischim Srl, Milan, Italy, unpublished report

WHO (World Health Organisation) (2009) Pesticide residues in food – 2009: toxicological evaluations / Joint Meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO Core Assessment Group, Geneva, Switzerland from 16 to 25 September 2009,
http://www.inchem.org/documents/jmpr/jmpmono/v2009pr01.pdf

Wilkinson CF, Killeen JC (1996) A mechanistic interpretation of oncogenicity of chlorothalonil in rodents and an assessment of human relevance. Regul Toxicol Pharmacol 24: 69–84

Wolf TM, Gallander KS, Downer RA, Hall FR, Fraley RW, Pompeo MP (1999) Contribution of aerosols generated during mixing and loading of pesticides to operator inhalation exposure. Toxicol Lett 105: 31–38

Zeneca (1998) A developmental toxicity study in rats via oral administration. Huntington Life Sciences, East Millstone, NJ, USA, Study number: 97–4112, Zeneca, Cheshire, England, unpublished report

Zeneca Agrochemicals (2000) Chlorothalonil technical: skin irritation study in rabbits. Central Toxicology Laboratory, Doc No. CTL/EB4891/REG/REPT, Zeneca Agrochemicals, Surrey, England, unpublished report

completed March 22, 2017