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INTEGRATED CRITERIA DOCUMENT CHLOROPHENOLS
EFFECTS

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CONTENTS

INTRODUCTION.....1

1 HUMAN TOXICITY.....3

1.1 Chemobiokinetics and metabolism.....3
1.1.1 PCP.....3
Animal data.....3
Oral exposure.....3
Exposure by inhalation.....6
Other routes of exposure.....6
Placental transfer.....7
Additional data.....7
Human data.....8
Oral exposure.....8
Autopsy data.....9
Additional data.....10
Additional data on PCP - animal and human data.....10
1.1.2 Chlorophenols other than PCP.....11
Animal data.....11
Human data.....12
1.1.3 Miscellaneous chlorophenols - animal and human data.....12
Summary and conclusions "chemobiokinetics and metabolism" 13
1.2 Toxicity.....15
1.2.1 Short-term exposure (acute and subacute toxicity).....15
Animal data - acute toxicity 15
Animal data - subacute toxicity.....16
Oral exposure.....16
Exposure by inhalation.....19
Human data - acute and subacute toxicity.....19
Summary and conclusions "short-term exposure" 20
1.2.2 Reproductive toxicity.....22
Oral teratology studies and reproduction studies.....22
Additional data.....28
Summary and conclusions "reproductive toxicity" 29
1.2.3 Long-term exposure (semichronic and chronic toxicity) - noncarcinogenic and carcinogenic effects.....31
Animal data.....31
Oral exposure.....31
Exposure by inhalation.....42
Human data.....43
Occupational exposure - non-carcinogenic effects.....43
Occupational exposure - carcinogenic and genotoxic effects.....45
Non-occupational exposure.....47
Summary and conclusions "long-term exposure" 48
1.2.4 Genotoxicity.....52
In vitro studies.....52
Gene mutations *in vitro* 52
Chromosomal aberrations *in vitro* 54
Other genotoxic effects *in vitro* 54
In vivo studies.....55
Additional data.....55
Summary and conclusions "genotoxicity" 56

Tables 1.1 to 1.6 (human toxicity).....58-93

2 ECOTOXICITY - I: AQUATIC ORGANISMS.....95

2.1 Accumulation.....95
2.2 Toxicity.....97
2.2.1 Freshwater organisms.....98
Short-term exposure ("single species" tests).....98
Long-term exposure ("single species" tests).....101
Field and ecosystem studies ("multiple species" tests).105
2.2.2 Marine organisms.....106
2.2.3 Relative toxicity chlorophenols (freshwater and marine organisms).....106
Summary and conclusions "aquatic organisms" 106

Tables 2.1 to 2.6 (aquatic toxicity).....108-124
List of abbreviations tables 2.1 to 2.6 125

3 ECOTOXICITY - II: TERRESTRIAL ORGANISMS.....127

3.1 Accumulation.....127
3.1.1 Plants (agricultural crops).....127
3.1.2 Earthworms - laboratory studies.....128
Accumulation from the soil.....128
Accumulation from solution.....129
3.1.3 Earthworms and other invertebrates - field studies.....129
3.2 Toxicity.....131
3.2.1 Microbe-mediated processes - laboratory studies.....131
3.2.2 Plants (agricultural crops) - laboratory studies.....131
3.2.3 Invertebrates - laboratory studies.....131
Earthworms.....131
Additional data.....133
3.2.4 Invertebrates - field studies.....134
Summary and conclusions "terrestrial organisms" 134

Table 3.1 to 3.4 (terrestrial toxicity).....138-142
List of abbreviations Tables 3.1 to 3.4 143

4 TOXICITY TO LIVESTOCK.....145

4.1 Chemobiokinetics and metabolism.....145
4.1.1 Poultry.....145
4.1.2 Mammals.....145
4.2 Toxicity.....147
4.2.1 Experimental studies.....147
4.2.2 Cases of intoxications.....151
Summary and conclusions "livestock" 152

5 RISK ASSESSMENT.....155

5.1 Risk assessment for man.....155
5.1.1 Oral exposure.....155
5.1.2 Exposure by inhalation.....156
5.2 Risk assessment for the environment.....156
5.2.1 Aquatic organisms.....157
5.2.2 Terrestrial organisms.....158

REFERENCES.....163

INTRODUCTION

Data in the present Appendix are underlying those in chapter 5 ("effects") in the "Integrated Criteria Document Chlorophenols" (Slooff et al., 1990). The Criteria Document, prepared by the National Institute of Public Health and Environmental Protection in the Netherlands, comprises a systematical survey and a critical evaluation of the most important data on chlorophenols, as much as possible with regard to the specific situation in the Netherlands. The information in the Criteria Document will serve as a scientific basis for an "effect oriented policy", especially with regard to the general population and aquatic and terrestrial ecosystems.
The Criteria Document, including the present Appendix, has been written by order of the Dutch Ministry for Housing, Physical Planning and Environment, Directorate Substances and Risk-management.

Data which are considered to be relevant to the risk assessment for man (general population) are described in chapter 1. Significant exposure can occur via oral intake and via inhalation. Outside occupational settings, dermal exposure is considered to be not relevant.
Data on the impact of chlorophenols on aquatic and terrestrial organisms are described in chapter 2 and chapter 3, respectively, and data on livestock in chapter 4.
In chapter 5, acceptable (exposure) levels for man, and for aquatic and terrestrial ecosystems are derived from the data reported in the aforementioned chapters.

An on-line literature search has been conducted early in 1989, in order to retrieve more recent publications (from 1980 and onwards). Additional publications originate from reviews and other publications.

1 HUMAN TOXICITY

1.1 CHEMOBIOKINETICS AND METABOLISM

The majority of data on kinetics and metabolism of chlorophenols refer to PCP. Therefore, data on PCP are discussed separately in section 1.1.1 and those on chlorophenols other than PCP have been clustered in section 1.1.2. The fate of PCP in several mammalian species, after oral exposure, has been investigated by Braun and co-workers in comparative studies with rats, monkeys and humans (Braun and Sauerhoff, 1976; Braun et al., 1977; Braun et al., 1979). In each of these studies, different aspects have been studied, namely absorption, metabolic transformation and excretion. Therefore, these aspects are not discussed in separate sections.

1.1.1 PCP

Animal data

Oral exposure

The kinetics and metabolism of ^{14}C-PCP were studied in three male and three female Sprague-Dawley rats. Following a single oral dose of 10 mg ^{14}C-PCP. kg^{-1} bw (in corn oil), the plasma PCP concentration increased rapidly; peak plasma concentrations of about 50 $mg.l^{-1}$ were reached 4-12 hours after administration, in both males and females. Subsequently, the PCP plasma concentration decreased according to a two-compartment kinetic model; in females the decrease was somewhat slower than in males. In the 8 days following administration of the 10 $mg.kg^{-1}$ bw dose, an average (3 males and 3 females) of 80% and 18.5% of the dose (% radioactivity) was excreted in urine and faeces, respectively; expired CO_2 only accounted for 0.2%. Only 0.44% of the dose was retained in the organs; of this amount, 70% and 10% was recovered in liver and kidneys, respectively. After administration of a single dose of 100 $mg.kg^{-1}$ bw, 64% and 34% of the dose was excreted in urine and faeces, respectively, in 9 days. At this dose level, the urine collected after 24 hours contained 75% unchanged PCP, 9% conjugated PCP (PCP glucuronide) and 16% tetrachloro-p-hydroquinone. Half-lives for the initial and terminal phase of elimination from the central compartment (as measured by urinary and faecal excretion) were calculated to be 13-17 and 40-120 hours, respectively, for male and female rats exposed to 10 $mg.kg^{-1}$

bw and male rats exposed to 100 $mg.kg^{-1}$ bw. In females exposed to 100 $mg.kg^{-1}$ bw, elimination from the central compartment was best described with a one-compartment kinetic model with a half-live of 27 hours. Both males and females excreted $\geq$ 90% of the dose within 3 days, independent of the dose level. In blood plasma, 99% of PCP was protein-bound at the low dose level. This high affinity for plasma proteins, together with reabsorption, explains the low renal clearance rate (Braun et al., 1977).
Using the kinetic parameters reported in this study with rats, Braun et al. (1979) calculated plasma parameters in rats, for a simulated single oral dose of 0.1 $mg.kg^{-1}$ bw. This resulted in a peak plasma concentration of 0.35 $mg.l^{-1}$, reached 4 hours after ingestion, and an absorption half-life of 0.4 hour. Clearance from the plasma was best described with a two-compartment kinetic model with elimination half-lives of 15 and 36 hours, for the initial and terminal phase, respectively. Further, it was calculated that in 7 days following ingestion, 80% of the simulated dose would be excreted in urine (75% unchanged PCP, 9% PCP glucuronide and 16% tetrachloro-p-hydroquinone) and 19% in the faeces (unchanged PCP). Braun et al. (1979) also calculated plasma parameters for a simulated repeated dose of 0.1 $mg.kg^{-1}$ $bw.day^{-1}$ for 7 days, followed by 7 days of recovery. In this case, 90% of the steady state plasma PCP concentration was reached in 1.5 days. The steady state concentration was calculated to be about 0.5 $mg.l^{-1}$, similar to the maximum plasma PCP concentration of 0.35 $mg.l^{-1}$ calculated for a single simulated dose of 0.1 $mg.kg^{-1}$ bw.

A comparison of plasma levels and kinetic parameters in rats after oral administration of NaPCP in drinking water (320 $mg.l^{-1}$) and after intravenous administration showed that virtually all the administed PCP was absorbed from drinking water (Anon, 1988).
After oral exposure of pregnant hamsters to daily doses of 1.25 to 20 mg $PCP.kg^{-1}$ bw on 6 consecutive days, the highest concentration in blood and fat was measured within 3 hours following the last dose administered. Concentrations in fat persisted in measurable amounts for a period of up to 5 days, and exceeded the concentration in blood at that time (Hinkle, 1973, abstract).
Following nasogastric intubation of a single dose of 10 mg ^{14}C-$PCP.kg^{-1}$ bw (in corn oil) in male and female rhesus monkeys, 3 animals of each sex, peak plasma PCP concentrations were reached 12-24 hours after administration. Both absorption and clearance showed first order kinetics. For males, half-lives of absorption and elimination were 3.6 and 72 hours, respectively. For females the corresponding values were 1.8 and 83 hours,

respectively. In the 15 days following exposure, the animals excreted 69%-78% of the dose (% radioactivity) in the urine and 12%-24% in the faeces; 8%-16% was retained in the tissues, especially in intestines and liver. Urine elimination half-lives were calculated to be 41 and 92 hours in males and females, respectively. Males excreted all activity in 7 days, while females excreted a considerable part of the activity after 7 days. According to the investigators, all the radioactivity in the urine was accounted for by unchanged PCP; metabolites were not detected (Braun and Sauerhoff, 1976).

Using the kinetic parameters reported in this study with monkeys, Braun et al. (1979) calculated plasma parameters in monkeys, for a simulated single oral dose of 0.1 $mg.kg^{-1}$ bw. This resulted in a peak plasma concentration of 0.1-0.3 $mg.l^{-1}$, reached 12-24 hours after ingestion, and an absorption half-live of 2.5 hour. Clearance from the plasma was best described with a linear one-compartment kinetic model with an elimination half-life of 78 hours. Further, it was calculated that in 15 days following ingestion, 70% and 18% of the simulated dose would be excreted in urine and faeces, respectively, as unchanged PCP. Braun et al. (1979) also calculated plasma parameters for a simulated repeated dose of 0.1 $mg.kg^{-1}$ $bw.day^{-1}$ for 7 days, followed by 7 days of recovery. In this case, 80% of the steady state plasma PCP concentration was reached at day 7, when exposure was terminated. The steady state plasma PCP concentration was calculated to be about 1.2 $mg.l^{-1}$.

In another study with rhesus monkeys, 2 males were exposed to a single oral dose of either 30 or 50 mg ^{14}C-PCP.kg^{-1} bw; one animal at each dose level was simultaneously exposed to cholestyramine, an ion exchange resin which depresses the enterohepatic circulation. In the 6 days following administration, animals solely exposed to either 30 or 50 mg ^{14}C-PCP.kg^{-1} bw excreted 26% and 15% of the total dose (which is less than the relative amount excreted in the former study with male rhesus monkeys exposed to a single oral dose of 10 mg ^{14}C-PCP.kg^{-1} bw). At 30 ^{14}C-PCP.kg^{-1} bw, urinary and faecal excretion accounted for 92% and 8%, respectively, of the total amount excreted. At 50 ^{14}C-PCP.kg^{-1} bw these figures were similar, 80% and 20%, respectively. Simultaneous exposure to cholestyramide increased the total amount excreted to 46% and 31% at 30 and 50 ^{14}C-PCP.kg^{-1} bw, respectively and reversed the elimination pattern from mainly urinary to predominantly faecal excretion. These results strongly indicate that absorbed PCP is mainly excreted via the bile but that enterohepatic circulation prevents faecal excretion (Ballhorn et al., 1981).

Exposure by inhalation

A single 20-minutes exposure of rats to an aerosol of NaPCP (total dose calculated: 5.7 mg PCP.kg^{-1} bw) resulted in a rapid absorption: immediately after exposure (t = 0), about 35%, 25% and 2% of the dose was detected in plasma, liver and lungs, respectively (In preliminary experiments, the kidneys and other tissues contained less than 2% and 0.5% (each), respectively. Therefore, these tissues were not analysed in this study). Clearance from the body also occurred rapidly: 24 hours after exposure, 50% of the dose was excreted in the urine. At this time, the liver, plasma and lungs acounted for 8%, 7% and 0.7% of the dose, respectively. After 72 hours, about 75% was excreted in the urine. At this time, the liver and plasma each contained less than 1% of the dose. The clearance rate of PCP from the liver was similar to that from the plasma, indicating no apparent accumulation in liver. In this inhalation experiment, only trace amounts of the metabolite tetrachloro-p-hydroquinone was detected in liver and urine, while in animals injected intraperitoneally (unpublished data Hoben et al.) about 50% of the injected dose was recoved as this metabolite. Repeated 20-minutes exposures to a similar concentration on 5 consecutive days tended to result in lower concentrations in plasma or liver and in an increase in the amount excreted in urine in 24 hours after exposure (from 55% to 70%). In addition, clearance from the tissues after the fifth exposure was very similar to that after a single exposure. These data show that PCP was not accumulated under the conditions of this test (Hoben et al., 1976b).

Other routes of exposure

A rapid absorption was observed in mice following intraperitoneal or subcutaneous injections of ^{14}C-PCP. In mice treated intraperitoneally with doses of 15-37 mg ^{14}C-PCP.kg^{-1} bw, 62%-83% and 4%-12% was excreted in urine and faeces, respectively, in 4 to 7 days. The urine of mice treated intraperitoneally with a single dose of 10 mg ^{14}C-PCP.kg^{-1} bw, contained 41% unchanged PCP, 13% conjugated PCP, 24% tetrachloro-p-hydroquinone, and 22% conjugated tetrachloro-p-hydroquinone. For rats, the corresponding values were 60%, 9%-16%, 7% and 16%-22%, respectively (WHO, 1987). In another study in which rats were treated intraperitoneally with 10 mg PCP.kg^{-1} bw (purity 99.9%), tetrachloro-p-hydroquinone was found to be the major metabolite also: during the first 24 hours after treatment, 45% and 48% of the amount present in the urine was found to be PCP and tetrachloro-p-hydroquinone. respectively. Trichloro-p-hydroquinone was detected as the

minor metabolite (7%). Pretreatment of rats with a single dose of either 2,3,7,8-tetrachlorodibenzo-p-dioxin (10 $\mu g.kg^{-1}$ bw, by gavage) or 3-methylcholanthrene (20 $mg.kg^{-1}$, intraperitoneally) strongly increased the dechlorination of PCP to tetrachloro-p-hydroquinone and slightly increased the dechlorination to trichloro-p-hydroquinone. The increased dechlorination of PCP to tetrachloro-p-hydroquinone was confirmed in *in vitro* studies with microsomes from pretreated rats. Pretreatment of rats with either 2,3,7,8-TCDD or 3-MC (which both are inducers of cytochrome P_1-450 [P-448]) doubled the amount of PCP equivalents excreted in the urine in 24 hours: 37% *versus* 75% (Ahlborg and Thunberg, 1978).

Placental transfer

To study placental transfer, a dose of 60 mg $PCP.kg^{-1}$ bw (^{14}C-PCP plus unlabeled-PCP) was orally administered to pregnant rats on day 15 of gestation. In blood (serum), the amount of labeled PCP and metabolites peaked 8 hours after dosing, at about 1% of the administered dose per gram of tissue. The amount in placentas and foetusses peaked 12 hours after dosing, at about 0.3% and 0.1%, respectively, indicating little placental transfer (Larsen et al., 1975). A contrasting result has been obtained in a preliminary study with only one monkey. Further data on this study are not available (WHO, 1987).

After oral exposure of pregnant hamsters to daily doses of 1.25 to 20 mg $PCP.kg^{-1}$ bw on days 5-10 of gestation, a close correlation between the concentrations in the maternal blood and entire foetuses was observed. As in maternal blood and fat, the highest concentration in foetuses was measured within hours following the last oral dose administered. (Hinkle, 1973, abstract).

Additional animal data on PCP

The distribution of PCP was investigated in a number of studies, using different laboratory animals and different routes of administration (oral, parenteral); animals were administered either a single dose, or repeated doses, which were $\geq$ 15 $mg.kg^{-1}$ bw ($.day^{-1}$). In these studies, the highest concentrations were usuallly found in the liver and kidneys (WHO, 1987).

Human data

Oral exposure

The kinetics and metabolism of NaPCP were studied in four male volunteers. Following ingestion of a single oral dose of 0.1 mg NaPCP.kg^{-1} bw (dissolved in water), the plasma PCP concentration increased rapidly, resulting in an average absorption half-life of 1.3 hours. Peak plasma concentrations (average value 0.2 mg.l^{-1}; maximum value 0.25 mg.l^{-1}) were reached after 4 hours. Control values were < 0.01 mg.l^{-1}. Subsequently, the plasma PCP concentration decreased linearly (according to a one-compartment kinetic model), resulting in an average plasma elimination half-life of 30 hours. The concentration of unmetabolized PCP in urine peaked after about 40 hours, after which the concentration decreased linearly, resulting in an average urine elimination half-live of 33 hours, equivalent to plasma elimination half-live. The concentration of metabolized PCP (PCP glucuronide) in urine peaked within 12 hours, after which the concentration also decreased linearly; this resulted in an average urine elimination half-life of 13 hours. In the 7 days following ingestion of the 0.1 mg.kg^{-1} bw dose, 86% (86% unchanged PCP and 14% PCP glucuronide) was excreted in the urine; 4% (50% unchanged PCP and 50% PCP glucuronide) was excreted in the faeces. Tetrachloro-p-hydroquinone and tetrachloro-p-hydroquinone glucuronide (known metabolites in the rat) could not be detected in urine. The fate of the remaining 10% was not determined. The lag time between the plasma peak concentration and the peak urinary concentration was ascribed to a strong enterohepatic recirculation similar to that reported in rats and monkeys. In addition, plasma parameters were also calculated for a simulated repeated dose of 0.1 mg.kg^{-1} bw.day^{-1} for 7 days, followed by 7 days of recovery. This daily dose is approximately equivalent to that received by workers exposed to a concentration of 0.5 mg.m^{-3} (500 μg.m^{-3}, the "Threshold Limit Value" in occupational settings) during a 8-hour work shift, assuming 100% retention. In this case, 90% of the steady state plasma PCP concentration was reached in 3.5 days; the steady state concentration was calculated to be about 0.5 mg.l^{-1}, reached in about 8 days. This steady state concentration was 2 times higher than the maximum concentration after a single oral dose of 0.1 mg.kg^{-1} bw (Braun et al., 1979).

In another study with male volunteers, (somewhat) different results were observed, especially with regard to elimination half-life. Following ingestion of a single oral dose (dissolved in 40% ethanol) of either 0.02

mg ^{13}C-PCP.kg^{-1} bw or 0.31 mg unlabeled-PCP.kg^{-1} bw (1 male per dose level), urine elimination half-lives of 18 and 20 days were calculated, respectively, based on first-order elimination kinetics. In the former experiment the plasma elimination half-life was calculated to be 16 days, similar to that in urine. More than 96% of plasma PCP was protein-bound which explains, together with reabsorption, the low renal clearance rate. In this experiment the concentrations of possible metabolites, viz. tetrachloro-p-hydroquinone, 2,3,4,5-T4CP and 2,3,4,6-T4CP, were below the limit of detection. In the latter experiment urinary PCP initially consisted of about 65% unchanged PCP and 35% PCP glucuronide. Two weeks after administration the amount of conjugated PCP was similar to that in non-specifically exposed persons, that is about 65%, although urinary excretion still was increased considerably (300 μg.day^{-1} versus 10-50 μg.day^{-1}). The theoretical amount of PCP excreted daily in urine, calculated on the basis of the renal clearance rate derived in the former experiment, was very similar to the detected amount. Therefore, elimination by other routes (faecal excretion, metabolism) is considered to be insignificant. The role of the enterohepatic circulation in elimination characteristics of PCP was investigated in an additional study with chlolelithiasis patients with postoperative T-drainage, in which PCP concentrations in plasma, bile and urine were compared. In this study no accumulation of PCP in the enterohepatic circulation was observed (Uhl et al., 1986). Literature data (reviewed by Uhl et al., 1986) on occupational exposed workers indicate elimination half-lives of 12 to 16 days.

Autopsy data

In victims of fatal intoxications resulting from different routes of exposure (oral, dermal, inhalation, or combined exposure: dermal/inhalation or dermal/oral), elevated PCP concentrations were mostly found in liver, kidneys and lungs. The concentrations in blood (mg.l^{-1} range) mostly were similar to those in the aforementioned organs (mg.kg^{-1} range), indicating a low accumulation potential in cases of acute intoxications.

In two studies concerning the general population, PCP concentrations in tissues and body fluids of persons without known exposure to PCP were analyzed. The results of these studies indicate, that there is only a slight tendency for PCP to accumulate in both liver and kidneys. No correlation between PCP concentrations in tissues and age was found (WHO, 1987).

Additional human data on PCP

In persons without known history of PCP exposure and in non-occupationally low-exposed persons, average urinary PCP levels (conjugates included) are about 15 and 50 $\mu g.l^{-1}$, respectively. Maximum levels in these persons are 20-30 and 100-150 $\mu g.l^{-1}$, respectively. In non-specifically exposed persons (n = 13), about two thirds of the total amount of PCP detected in the urine was found to be conjugated to glucuronic acid. Non-occupational exposure to elevated indoor PCP levels of ≤ 5 and > 5 $\mu g.l^{-1}$ (frequently 5-10 $\mu g.m^{-3}$; exceptionally 10-25 $\mu g.m^{-3}$) due to the application of wood preservatives resulted in median urinary PCP levels of 25-50 and 40-80, respectively, in different groups of persons. In occupationally exposed persons, urinary PCP levels can be much higher; in heavily exposed workers these levels are in the $mg.l^{-1}$ range. Blood, plasma, or serum PCP levels are of the same order of magnitude as those in urine: up to about 50 and 500 $\mu g.l^{-1}$ in persons without known exposure and in non-occupationally exposed persons, respectively and 1-10 $mg.l^{-1}$ in (heavily) exposed workers. In cases of obvious intoxications these levels are > 40 $mg.l^{-1}$ (Klemmer et al., 1980; Krause and Englert, 1980; Sangster et al., 1982; Uhl et al., 1986; WHO, 1987).

Several animals species have been found to metabolize hexa- and penta-chlorobenzene to PCP and tetrachloro-p-hydroquinone. Therefore, the levels of these compounds in tissues and excreta do not necessarily reflect exposure to PCP itself (Koss and Koransky, 1978).

There are indications that tetrachloro-p-hydroquinone may be formed as a (minor) metabolite of PCP in humans, but these indications are based on mixed exposures to PCP and other chlorophenolic compounds and on an *in vitro* study using human liver homogenates (WHO, 1987).

Additonal data on PCP - animal and human data

Lilienblum (1985) showed that PCP glucuronide is stable at neutral pH for several hours, but that considerable hydrolysis occurs under the weak acidic conditions normally observed in urine. Therefore, measurements of urinary PCP glucuronide may underestimate the portion actually conjugated before excretion. This author also compared the glucuronosyltranferase activity toward PCP in rat liver microsomes and human liver microsomes; the activity in the former was about 3 times higher than that in the latter (Lilienblum, 1985).

1.1.2 Chlorophenols other than PCP

Animal data

There are relatively few studies on the fate of chlorophenols other than PCP in mammals. Much of the information is based on studies in which kinetics and metabolism of chlorophenols formed metabolically from other organochlorine compounds (such as lindane which is metabolized to di-, tri- and tetrachlorophenols) have been studied. The data in this section are based primarily on the "Environmental Health Criteria Document" on chlorophenols other than PCP (WHO, 1989).

After daily intragastrical administrations to rats of 50 or 100 mg 2,3,4,6-T4CP.kg^{-1} bw (in olive oil) for 8 weeks, the highest concentrations were recovered in kidneys (1 and 5 mg.kg^{-1} bw, respectively) and spleen (1.4 and 3.2 mg.kg^{-1} bw, respectively); the concentrations were lowest in muscle and brain. At a daily dose of 10 mg.kg^{-1} bw the concentrations in kidneys and spleen were "very low" (Hattula et al., 1981b). After a single parenteral administration to rats of 2,4-DCP (i.v.) or 2,4,6-T3CP (i.p.), the highest concentrations were also found in the kidneys; in addition, relatively high concentrations were found in the liver. In dietary studies with livestock, the distribution of chlorophenols as metabolites of other compounds was investigated. In cattle and sheep fed 2,4-dichlorophenoxyacetic acid (2,4-D) for 28 days, the highest and next highest 2,4-DCP concentration were found in the kidneys and liver, respectively, in both species. In cattle and sheep fed trichlorophenoxy acid herbicides for 28 days, the highest and next highest 2,4,5-T3CP concentrations were found in the liver and kidneys, respectively, in both species (WHO, 1989).

In two oral studies in which rats were administered either a single dose or 3 daily doses of ^{14}C-2,4,6-T3CP, at least 80% of the total dose was excreted in the urine, within 1-7 days; 5%-20% was excreted in the faeces. Similar results (based on studies with laboratory animals and livestock) were reported after parenteral administration of 2,4,6-T3CP or other compounds, both chlorophenols and other organochlorine compounds. There are indications that the clearance from organs such as liver and kidneys of chlorophenols (metabolically formed from other compounds) is slower than their elimination via the urine (WHO,1989).

With regard to metabolic transformations it has been found in a number of studies using different laboratory animals, different routes of exposure and different compounds, that the lower chlorinated chlorophenols (MCP, DCP, T3CP) are present in tissues and body fluids mainly as glucuronide and

sulfate conjugates, both after administration of chlorophenols and other organochlorine compounds (WHO, 1989). One study is available on the metabolism of the different isomers of T4CP; in this study rats were injected intraperitoneally with a dose level of 10 $mg.kg^{-1}$ bw of the respective isomer. After administration of 2,3,5,6-T4CP, 33% and 66% of the dose administered was excreted in the 0-24 hr urine as tetrachloro-p-hydroquinone and as parent compound (and/or conjugates), respectively. After administration of 2,3,4,5-T4CP and 2,3,4,6-T4CP, 51% and 94% of the dose administered was excreted in the 24-hr urine, respectively. The latter two compounds were excreted essentially unchanged or as conjugates; trichloro-p-hydroquinone was found to be a minor metabolite. In this study the urine was boiled with concentrated hydrochloric acids; therefore, no distinction between parent compounds and conjugates could be made. (Ahlborg and Larsson, 1978). [It is noted that the dose level reported in this study, 10 $mg.kg^{-1}$ bw, does not correspond with the reported total dose of 4.9 to 5.3 mg for rats with a body weight of 200-300 g]

Human data

For 2,4,5-T3CP, urinary levels ranging from <5 to 30 $\mu g.l^{-1}$ have been reported in persons without known history of exposure (Ahlborg and Thunberg, 1980). In groups of sawmill workers exposed to tetrachlorophenols (sodium salts) mean and median urinary T4CP levels ranged from 160 to 2,840 $\mu g.l^{-1}$; maximum levels in these groups of workers ranged from 1,5 to approximately 50 $mg.l^{-1}$ (WHO, 1989).

1.1.3 Miscellaneous chlorophenols - animal and human data

Quantitative data on the absorption of PCP and other chlorophenols at dermal exposure or exposure by inhalation are hardly available. However, animal data (especially acute toxicity studies), human data (the appearance of a variety of local and systemic effects due to exposure to PCP and other chlorophenols, especially in occupational settings) and *in vitro* experiments using mammalian skin indicate that chlorophenols are "readily" absorbed via these routes of exposure. Absorption (through the skin) occurs especially when the compounds are in the un-ionized form, i.e. at pH-value below pKa-value (WHO, 1987, 1989).

The accumulation of 2-MCP and PCP in liver and kidneys was determined in a reproduction study in which groups of female rats were exposed from 3 weeks

of age through gestation (bred at 90 days) and lactation to concentrations of 0, 5, 50 and 500 mg 2-MCP.l^{-1} drinking water (equivalent to 0, 0.5, 5 and 50 mg 2-MCP.kg^{-1} bw.day^{-1}) or 0, 5, 50 and 500 mg PCP.kg^{-1} feed (equivalent to 0, 0.25, 2,5 and 25 mg PCP.kg^{-1} bw.day^{-1}). In animals exposed to 2-MCP, the concentration in livers of low- and mid-dosed animals was 14 and 20 times higher than that of control animals; that in high-dosed animals was 2 times lower than that of control animals. The concentration in kidneys of animals exposed to 2-MCP was 8- to 10-times higher than that of control animals; there was a trend of decreasing concentrations of 2-MCP in kidneys with increasing dose levels, but the differences were small. In the study with PCP, the concentration in the livers of exposed animals was about 2 times higher than that of control animals and that in kidneys was (somewhat) lower than that of control animals, at all dose levels tested. The concentrations of 2-MCP in both liver and kidneys were consistently higher than that of PCP, both in control and dosed animals (Exon and Koller, 1982).

Summary and conclusions "chemobiokinetics and metabolism"

PCP

The fate of PCP after oral exposure has been investigated in comparative studies with a limited number of rats, monkeys and humans. These studies show that PCP is absorbed rapidly and (approximately) completely from the gastrointestinal tract, after a single dose of either 0.1 mg.kg^{-1} bw (humans) or 10 mg.kg^{-1} bw (animals). For a single oral dose of 0.1 mg.kg^{-1} bw, absorption half-lives of 0.4, 1.3 and 2.5 hours were calculated for rats, humans and monkeys, respectively. Elimination half-lives were calculated to be 15, 30 and 78 hours for rats, humans and monkeys, respectively. For humans it was calculated that repeated exposure to a dose level of 0.1 mg.kg^{-1} bw.day^{-1} will result in a steady-state PCP plasma concentration after about 8 days. Based on these data it appears that the accumulation of PCP will be limited at repeated exposure to similar dose levels. However, other experimental data and data on occupational exposed workers indicate elimination half-lives of approximately 15 days for humans.

Both animal and human studies show that PCP is excreted primarily in the urine ($\geq$ 65%). Rats excrete PCP primarily as parent compound (75%) and the remaining part as PCP glucuronide conjugate and as tetrachloro-p-hydroquinone (TCH), in similar amounts. In humans a higher percentage of

PCP is conjugated before excretion (35%-65%), especially at low exposure levels. Neither humans nor monkeys metabolize PCP into TCH; monkeys excrete PCP essentially unchanged. It is noted that the reported percentages for urinary excretion and metabolism are not absolute, but depent on exposure level.

The comparative oral studies show sufficient similarity between the rat and man with regard to most parameters studied, to consider the rat as a useful animal model to study the fate of PCP in man.

In both animals and humans, the highest concentrations are usually observed in the liver and kidneys. In persons without known history of PCP exposure and in non-occupationally low-exposed persons, total-PCP concentrations in blood and urine usually are 10-100 (up to 500) $\mu g.l^{-1}$. In heavily exposed workers these concentrations are in the (low) $mg.l^{-1}$ range.

Limited data and physico-chemical properties indicate that PCP is also readily absorbed at dermal exposure and, especially, at exposure by inhalation.

Chlorophenols other than PCP

Data on these compounds are much more limited than those on PCP. Based on the available data and the physico-chemical properties of chlorophenols it is assumed that all chlorophenols are readily absorbed and excreted, urine being the major route of elimination. In tissues and body fluids, lower-chlorinated compounds (MCP, DCP, T3CP) are present primarily as glucuronide and sulfate conjugates. In rats, TCH may be a major metabolite of 2,3,5,6-T4CP, while the other two T4CP isomers are excreted essentially unchanged or as conjugates.

In animal studies with miscellaneous chlorophenols, the highest concentrations were observed in the liver, kidneys (and spleen).

1.2 TOXICITY

1.2.1 Short-term exposure (acute and subacute toxicity)

Most signs and symptoms at lethal exposure to different chlorophenols are similar, and include motor weakness, an increase in respiration rate and body temperature, tremors, CNS depression, convulsions, dyspnea, and coma. However, there are differences which are dependent on the degree of chlorination. The occurence of convulsions is associated especially with the lower chlorinated phenols; this effect is ascribed to the undissociated molecule. Uncoupling of oxidative phosphorylation, resulting in metabolic effects such as increases in respiration rate and body temperature, is associated especially with the higher chlorinated phenols, notably PCP; the uncoupling effect is ascribed to the chlorophenate ion. In rat liver mitochondria the uncoupling effect of PCP was found to be 40 times greater than that of 2,4-DCP. The uncoupling effect of PCP in human microsomes was found to be 10 times greater than that in rat microsomes (Ahlborg and Thunberg, 1980; Exon, 1984; Borzelleca et al., 1985c; WHO, 1987).

Animal data - acute toxicity

Acute LD50- and LC50-values are summarized in table 1.1. Most data in this table are from secundary literature sources (WHO, 1987, 1989; RTECS 1989). At oral exposure, LD50-values for MCP, DCP, T3CP, T4CP and PCP are 260-1,400, 465-4,000, 455-2,960, 90-980 and 25-295 $mg.kg^{-1}$ bw, respectively; those for NaPCP are 70-700. These values are based on different studies. Therefore, the variations found for one compound or for one group of isomers (e.g. T3CP are, at least in part, the result of differences in experimental procedure (animal species, strain, age, vehicle, purity test compound). Dermal, subcutaneous or intraperitoneal exposure has resulted in LD50-values which are in many cases within a factor of 2 compared with those after oral exposure. Exposure by inhalation resulted in LC50-values of 11 $mg.m^{-3}$ for 4-MCP and of 225-355 $mg.m^{-3}$ for (Na)PCP. LC50-values for other chlorophenols are not available.

When NaPCP was administered orally to rats or rabbits, or dermally to rabbits, the lowest LD50-value was 3 to 5 times higher than the corresponding LD50-value for PCP. When NaPCP was administered dermally, subcutaneously or intraperitoneally to rats, or subcutaneously to mice or rabbits, LD50-values were similar (within a factor of 2) to those for PCP.

The LD50 of 12 $mg.kg^{-1}$ bw for inhaled NaPCP (Hoben et al., 1976a) is at least 6 times lower than oral LD50-values based on tests with the same species (rat).

Animal data - subacute toxicity

Oral exposure

Oral, subacute toxicity studies are summarized in table 1.2. If more than one study was available for one compound, the studies are listed in the following order: i) animal species (from "small" to "large"), ii) exposure time, (from "short" to "long") and iii) purity of test compound (from "high purity" to "low purity"). For each study, a lowest-effect-dose, LED, and a no-observed-(adverse)-effect-level, NO(A)EL, are mentioned in the table, if possible. These values have been based on an evaluation of the data reported and do not necessarily represent the opinion of the investigators. Most of these studies were "range finding" experiments for teratology studies or (semi)chronic toxicity studies. Effects on survival, body weight, organ weights, and, additionally, gross pathology and histopathology are considered to be the most relevant endpoinds in subacute toxicity studies. Therefore, especially the effects on these parameters are discussed in the text below and used to derive the LED and NO(A)EL. Furthermore, "target" organs (organs which were affected at the LED or at higher dose levels) are reported.
In the text below, dose levels are expressed as $mg.kg^{-1}$ $bw.day^{-1}$, regardless of treatment procedure.

2-MCP

Exposure by gavage of mice for two weeks to 2-MCP (purity not reported) resulted in mortality at 175 $mg.kg^{-1}$ $bw.day^{-1}$ and in reduced body weights at 69 $mg.kg^{-1}$ $bw.day^{-1}$; a dose of 35 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect (Borzelleca, 1985c)

2,4-DCP

Exposure of mice for 2 weeks to 2,4-DCP (purity > 99%) in feed, did not result in an effect on body weight gain at 2,800 $mg.kg^{-1}$ $bw.day^{-1}$, although feed intake was strongly reduced at this dose level. Therefore,

2,800 mg.kg^{-1} bw.day^{-1} is considered to be the lowest-effect-dose. At 1,400 mg.kg^{-1} bw.day^{-1}, both weight gain and feed intake were similar to controls; this dose level is considered to be without effect (NTP, 1989a). Exposure by gavage of mice for two weeks to 2,4-DCP (purity not reported) at dose levels up to 638 mg.kg^{-1} bw.day^{-1} did not result in an effect on most parameters studied, including mortality, body and organ weights and gross pathology. Therefore, this dose level is considered to be without effect (Borzelleca, 1985c).
Exposure of rats for 2 weeks to 2,4-DCP (purity > 99%) in feed resulted in reduced feed intake and reduced body weight gain at 2,000 mg.kg^{-1} bw.day^{-1}; a dose of 1,000 mg.kg^{-1} bw.day^{-1} was without effect (NTP, 1989a).

2,4,5-T3CP

A 3-week study in which rats were given by stomach tube 18 doses of 2,4,5-T3CP (purity 97%-98%) in 24 days, resulted in a 15% increase in the weight of kidneys at 750 mg.kg^{-1} bw.day^{-1}; a dose of 225 mg.kg^{-1} bw.day^{-1} was without effect. In a similar study with rabbits, "very slight kidney changes" and "very slight kidney and liver changes" were reported at 70 and 350 mg.kg^{-1} bw.day^{-1}, respectively. Because no further data on these changes were reported, and because of the very low number of experimental animals, this study cannot be evaluated (McCollister et al., 1961).

2,4,6-T3CP

Exposure of mice and rats for 7 weeks to 2,4,6-T3CP (purity 96%-97%) in feed, resulted in a reduced body weight gain at 2,100 and 1,470 mg.kg^{-1} bw.day^{-1}, respectively. A dose of 1,400 and 1,000 mg.kg^{-1} bw.day^{-1} was without effect. Target organs in rats were spleen and liver; target organs in mice were not reported (NCI, 1979).

2,3,4,6-T4CP

Oral exposure (no further data) of female rats for 10 days to "commercial-grade" 2,3,4,6-T4CP (purity 73%) resulted in increased mortality at 100 mg.kg^{-1} bw.day^{-1}; a dose of 30 mg.kg^{-1} bw.day^{-1} was without effect (Schwetz et al., 1974a). In a study in which rats were exposed intragastrically for 8 weeks to 2,3,4,6-T4CP (purity > 99%), severe histopathological changes (e.g. necroses) were observed in the liver at 50 mg.kg^{-1} bw.day^{-1}; at 100

mg.kg^{-1} bw.day^{-1} the small intestine was also affected. A dose of 10 mg.kg^{-1} bw.day^{-1} was without effect (Hattula et al., 1981b).

PCP

The effect of two different grades of PCP, namely "Dowicide EC-7" (a relatively low-impurity grade) and "technical-grade" PCP (a relatively high-impurity grade), on the *in vivo* antibody response was investigated in a comparative study in which female mice were exposed by daily gastric intubations for 2 weeks. Exposure to "technical-grade" PCP at dose levels of 10, 30 and 100 mg.kg^{-1} bw.day^{-1} resulted in a dose-related decrease in antibody response after immunization with sheep red blood cells; the decrease was statistically significant at all dose levels. On the contrary, exposure to "Dowicide EC-7" at a dose level of 100 mg.kg^{-1} bw.day^{-1} did not result in a decreased antibody response (Holzapple et al., 1987).
In a comparative toxicity study, mice were exposed for 4 weeks to "pure" PCP (purity 98.6%), "Dowicide EC-7" (91% PCP) or "technical-grade" PCP (purity 90%) in feed. In all three studies, the effects with regard to mortality, weight gain and histopathological changes (observed in the liver) were identical, although minor quantitative differences in toxicity of the compounds were observed with respect to mortality and the number of animals with histopathological liver lesions. Based on the most sensitive parameter (liver lesions), all studies resulted in a LED of 70 mg.kg^{-1} bw.day^{-1}; a dose of 14 mg.kg^{-1} bw.day^{-1} was without effect (NTP, 1989b).
[In these experiments, supplemental parameters such as liver enzymes (aryl hydrocarbon hydroxylase, cytochrome P450) have been studied. Because of the relative short exposure time, the limited reporting of the results and the fact that these parameters were not included in other subacute studies, these parameters were left out of consideration. For the effect of different grades of PCP on these parameters, the reader is referred to the section on long-term exposure.]
Oral exposure (no further data) of female rats for 10 days to "commercial-grade" PCP (Purity 88%) resulted in weight loss at 70 mg.kg^{-1} bw.day^{-1}; a dose of 50 mg.kg^{-1} bw.day^{-1} was without effect (Schwetz et al., 1974b).
In a study in which rats were exposed for 8 weeks to "pure" PCP (purity > 99%) in feed, no effect on mortality, body weight gain and liver weight was observed at 40 mg.kg^{-1} bw.day^{-1}; other dose levels were not included in this study (Debets et al., 1980). *[For data on supplemental parameters (microsomal liver enzymes; urinary porphyrins) studied by Debets et al., see table 1.2]*

Exposure by inhalation

Short-term animal data on PCP and other chlorophenols are not available.

Human data - acute and subacute toxicity

The present section is based on the "Environmental Health Criteria Documents" on PCP (WHO, 1987) and on chlorophenols other than PCP (WHO, 1989).
There appear to be no studies or case reports on the effects of "pure" chlorophenols on humans. Therefore, the effects described below may be influenced by the impurities present in the formulations used. However, the early onset of morbidity and mortality at exposure to high concentrations, are most probably caused by the chlorophenols, not by the impurities.

PCP

Common signs and symptoms of acute toxicity are well known, based on numerous case reports on accidental or suicidal poisoning incidents (many of which have resulted in death) with "commercial-grade" PCP. These signs and symptoms include ataxia, mental and physical fatique, heachache, dizziness, disorientation, anorexia, nausea, vomiting, dyspnoea, hyperpyrexia, tachycardia, and a rise of metabolic rate. Weakness, elevated body temperature and profuse sweating are most prominent. In lethal cases, death is due to cardiac arrest, and victims usually show a marked rigor mortis. The minimum lethal oral dose has been estimated to be 30 $mg.kg^{-1}$ bw. In contrast to the lower chlorinated phenols, PCP does not cause convulsions.
Gross pathology and histological lesions are generally consistent with those observed in animal studies. Gross lesions include hepatomegaly, splenomegaly and cardiomegaly, and renal and hepatic congestion. Histological lesions include fatty degeneration and necrosis of the liver, and degenerative lesions in renal tubules. After oral exposure, gastric and intestinal inflammation has been reported. Pulmonary oedema and congestion have been reported after exposure by inhalation and sometimes after oral exposure, if aspiration has occured (WHO, 1987).
Indoor exposure to elevated levels in air, resulting from the application of PCP in the interior of houses, has resulted in (sub)acute non-specific signs and symptoms of poisoning which are similar to those observed in

poisoning incidents and in occupational settings (WHO, 1987). For example, case histories of 15 members of 3 families in the Netherlands, exposed in treated houses to airborne levels of 0.2 to 1.2 $\mu g.m^{-3}$ for 10 days to 8 months, show one or more of the following effects: burning sensation in the unprotected skin, similar reaction in the throat, dryness and scaling of face and hands, slight erythema, nausea, vomiting, decreased appetite, headache, dizziness and fatique. It can not be concluded whether these effects were caused by PCP itself or by the vapours origination from the organic solutions used. In three of these persons exposed for 8 months to concentrations up to 0.25 $\mu g.m^{-3}$, a 2- to 3-fold increase in plasma PCP concentrations was observed. Plasma PCP concentrations in these and the other exposed persons ranged from 25 to 660 $\mu g.l^{-1}$; these values were in the same range as those measured in 99 non-selected Dutch male draftees (<50 to 1,100 $\mu g.l^{-1}$; mean value 130 $\mu g.l^{-1}$; 95% range 330 $\mu g.l^{-1}$). Routine haematological, biochemical and urine analyses showed no abnormalities in the exposed persons (Sangster et al., 1982). In another investigation among non-occupational exposed persons, exposed to PCP- and lindane-containing wood preservatives, similar subacute effects were reported (Janssens and Schepens, 1985). For data on repeated exposure in occupational settings, the reader is referred to the section on long-term exposure.

Chlorophenols other than PCP

Known signs and symptoms of chlorophenols other than PCP are based primarily on animal studies. Occupational exposure has been most consistently associated with effects such as irritation of skin and mucous membranes and with chloracne (WHO, 1989).

Summary and conclusions "short-term exposure"

Animals

Oral LD50-values for MCP, DCP, T3CP, T4CP and PCP are 260-1,400, 465-4,000, 455-2,960, 90-980, and 25-295 $mg.kg^{-1}$ bw, respectively; for NaPCP these values are 70-700 $mg.kg^{-1}$ bw. These values show that T4CP and, especially, PCP are considerably more toxic than the lower-chlorinated compounds. Dermal, subcutaneous or and intraperitoneal exposure has resulted in LD50-values which are in many cases within a factor of 2 compared with those after oral exposure. Exposure by inhalation resulted in LC50-values of 11 $mg.m^{-3}$ for 4-MCP, and 255-355 $mg.m^{-3}$ for (Na)PCP. An inhalation study with

NaPCP resulted in a LD50-values of 12 $mg.kg^{-1}$ bw, which is 6 times lower than the lowest oral LD50-value for this compound.

Oral, subacute toxicity studies, exposure time 10 days to 8 weeks, are available for a limited number of chlorophenols; most studies refer to 2,4-DCP or PCP. Based on parameters such as survival, body and organ weights, and (histo)pathology, the following NO(A)ELs have been derived: 35 $mg.kg^{-1}$ $bw.day^{-1}$ for 2-MCP, 640-1,400 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4-DCP, 225 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4,5-T3CP, 1,000-1,400 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4,6-T3CP, 10-30 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,3,4,6-T4CP and 14-50 $mg.kg^{-1}$ $bw.day^{-1}$ for PCP. These data also show a trend of increasing toxicity with chlorination (consistent with acute toxicity values). It is noted that this conclusion is based on a limited number of data which are not similar for each compound studied.

In the studies with T3CP and the higher-chlorinated compounds, histo(patho)logical changes were observed in the liver; in the study with 2,4,5-T3CP changes were also observed in the kidneys.

Humans

Signs and symptoms of acute toxicity of PCP are well known, based on numerous case reports on accidental or suicidal poisoning incidents (with "commercial-grade" PCP). Metabolic effects such as an increase in respiration rate, elevated body temperature and profuse sweating are most prominent effects, consistent with the uncoupling of oxidative phosphorylation. Gross pathology and histological lesions observed in cases of poisoning are primarily related to the liver, consistent with the results in animal studies. Non-occupational exposure to airborne PCP concentrations of 0.2 to 1.2 $\mu g.m^{-3}$, resulting from the application of PCP in the interior of houses, has resulted in non-specific effects (such as irritation of skin and mucous membranes, nausea, vomiting, headache, dizziness and fatigue) which are similar to those observed in poisoning incidents and occupational settings.

Data on chlorophenols other than PCP are based primarily on acute toxicity studies with experimental animals. Occupational exposure has been most consistently associated with effects on skin and mucous membranes.

1.2.2 Reproductive toxicity

Oral teratology studies and reproduction studies

Data available include (short-term) teratology studies, mostly conducted in accordance with the protocol for so-called "segment II" studies, and (long-term) reproduction studies. In the text below the teratology studies are decribed more in detail than the reproduction studies, because the latter studies are also summarized in table 1.3. For each reproduction study, a lowest-effect-dose, LED, and a no-observed-(adverse)-effect-level, NO(A)EL, are mentioned in the table, if possible. These values have been based on an evaluation of the data reported and do not necessarily represent the opinion of the investigators.
In a number of the reproduction studies, effects on the progeny (parameters: postnatal survival and growth, organ weights, haematology and immunocompetence) have been studied in addition to reproductive performance (including parameters such as fertility, litter size, number of stillborn and birth weight) of the parental generation. In the teratology studies the animals were treated by gavage; in the reproduction studies, the parent animals were exposed either via feed or via drinking water.

2-MCP

A reproduction study in which female rats were exposed to 2-MCP (purity 97%) in drinking water, from 3 weeks of age through gestation, resulted in a decreased litter size and in an increased number of stillborn at 500 $mg.l^{-1}$ (equivalent to 50 $mg.kg^{-1}$ $bw.day^{-1}$). A concentration of 50 $mg.l^{-1}$ (equivalent to 5 $mg.kg^{-1}$ $bw.day^{-1}$) was without effect on reproductive performance. Maternal toxicity was not observed at any concentration tested. An extention of the exposure of the dams through lactation, followed by exposure of the progeny for an additional 10-15 weeks, did not result in effects on the progeny exposed both pre- and postnatally, at any concentration tested. A second part of this study is reported in section 1.2.3, "long-term exposure" (Exon and Koller, 1982, 1983a,b, 1985; table 1.3 and 1.4).

2,4-DCP

In a fertility and reproduction study in which adult male and female mice were exposed to 2,4-DCP (purity 99%) in drinking water for 3 months before

mating and additionally throughout mating and gestation, concentrations up to 2,000 $mg.l^{-1}$ (equal to 385 and 490 $mg.kg^{-1}$ bw.day for males and females, respectively) did not result in an effect on fertility and on reproductive performance (Borzelleca et al., 1985b,c, table 1.3).
A reproduction study in which female rats were exposed to 2,4-DCP (purity 99%) in drinking water, from 3 weeks of age through gestation, resulted in a decreased litter size at 300 $mg.l^{-1}$ (equivalent to 30 $mg.kg^{-1}$ $bw.day^{-1}$). A concentration of 30 $mg.l^{-1}$ (equivalent to 3 $mg.kg^{-1}$ $bw.day^{-1}$) was without effect on reproductive performance. Maternal toxicity was not observed at any concentration tested. Additionally, exposure of the dams to 300 $mg.l^{-1}$ resulted in an increase in spleen weight of the progeny exposed only prenatally, while a dose level of 30 $mg.l^{-1}$ did not affect prenatally exposed progeny. An extention of the exposure of the dams throughout lactation, followed by exposure of the progeny for an additional 10-15 weeks, resulted in effects on the progeny: pre-and postnatal exposure resulted in a decreased DTH-response at 30 and 300 $mg.l^{-1}$, and in an increase in antibody production and increased spleen and liver weights at 300 $mg.l^{-1}$. A concentration of 3 $mg.l^{-1}$ (equivalent to 0.3 $mg.kg^{-1}$ $bw.day^{-1}$) was without effect, with regard to all parameters studied. A second part of this study is reported in section 1.2.3, "long-term exposure (Exon et al., 1984; Exon and Koller, 1985; table 1.3 and 1.4).

In a teratology study ("segment II" study) the embryo/foetotoxicity and teratogenicity of "technical-grade" 2,4-DCP (purity 99.2%; dibenzo-p-dioxins not found) were studied in rats. Groups of 34 Fischer 344 rats were treated by gavage with doses of 0 (vehicle control), 200, 375 or 750 $mg.kg^{-1}$ $bw.day^{-1}$ (in corn oil) from day 6 through day 15 of gestation. Animals were killed on day 20 of gestation. At 750 $mg.kg^{-1}$ $bw.day^{-1}$, 4 animals died during the treatment period. Maternal body weight gain was dose-related decreased during the treatment period; this effect was statistically significant ($p < 0.05$) at all dose levels tested, although body weight gains at 200 and 375 $mg.kg^{-1}$ $bw.day^{-1}$ were comparable to that of control animals. With regard to reproductive performance no statistically significant effects were found at termination, although the number of resorptions was somewhat increased at 750 $mg.kg^{-1}$ $bw.day^{-1}$. With regard to external, soft tissue and skeletal variations, the number of foetusses and litters with delayed ossification of sternabrae-numbers 1, 2, 3 and/or 4 or vertebral arches was increased (statistically significant at the level of litters) at 750 $mg.kg^{-1}$ $bw.day^{-1}$. At this dose level, the incidences of these variations in foetusses (and litters) were 4/80 (4/22)

and 6/80 (6/22), respectively, while these effects were not observed in control foetusses (Rodwell et al., 1989).

2,4,6-T3CP

A reproduction study in which female rats were exposed to 2,4,6-T3CP (purity > 99%) by gavage from 2 weeks prior to mating throughout gestation, resulted in severe maternal toxicity (including mortality) at 1,000 $mg.kg^{-1}$ $bw.day^{-1}$. A dose level of 500 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect with regard to maternal toxicity, reproductive performance and effects on the progeny. A study in which male rats were exposed to 2,4,6-T3CP (purity > 99%) by gavage for 11 weeks prior to mating with untreated females, resulted in severy paternal toxicity (including mortality) at 1,000 $mg.kg^{-1}$ $bw.day^{-1}$. A dose level of 500 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect with regard to paternal toxicity, and male and female reproductive performance (Blackburn et al., 1986; table 1.3).
A reproduction study in which female rats were exposed to 2,4,6-T3CP (purity 98%) in drinking water, from 3 weeks of age through gestation, resulted in a decreased litter size at 300 $mg.l^{-1}$ (equivalent to 30 $mg.kg^{-1}$ $bw.day^{-1}$); a concentration of 30 $mg.l^{-1}$ (equivalent to 3 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect on reproductive performance. Data on maternal toxicity are not reported. An extension of the exposure of the dams through lactation, followed by exposure of the progeny for an additional 12 weeks, resulted in effects on the progeny: pre-and postnatal exposure resulted in an increased liver weight at 30 $mg.l^{-1}$, and in increased liver and spleen weights at 300 $mg.l^{-1}$. A concentration of 3 $mg.l^{-1}$ (equivalent to 0.3 $mg.kg^{-1}$ $bw.day^{-1}$) was without effect with regard to all parameters studied (Exon and Koller, 1985; table 1.3).

2,3,4,6-T4CP

In a teratology study ("segment II" study) the embryo-/foetotoxicity and teratogenicity of two grades of 2,3,4,6-T4CP were studied in rats. Groups of 20 Sprague-Dawley rats were treated by gavage with doses of 0 (vehicle control), 10 or 30 $mg.kg^{-1}$ $bw.day^{-1}$ (in corn oil) from day 6 through day 15 of gestation. The highest dose level tested was the highest dose without signs of toxicity in a preliminary 10-d tolerance study (table 1.2). Animals were killed on day 21 of gestation. The two grades of this compound were "commercial-grade" (purity 73%; 27% PCP; 2,3,7,8-TCDD <0.05 ppm, 28 ppm HCDD, 80 ppm HpCDD, 30 ppm OCDD, 55 ppm HCDF, 100 ppm HpDCF,

25 ppm OCDF) and "purified" (purity 99.6%; 0.1% PCP; 2,3,7,8-TCDD <0.05 ppm, HCDD <0.5 ppm, HpCDD <0.5 ppm, OCDD <0.5 ppm, HCDF <0.5 ppm, HpCDF <0.5 ppm, OCDF <0.5 ppm).

Signs of maternal toxicity were not observed at any dose of either compound. The number of resorbed foetuses, sex ratio, foetal body weight and foetal crown-rump length were affected neither. At 30 $mg.kg^{-1}$ $bw.day^{-1}$, the incidence of delayed ossification of the skull bones among foetuses (17%, 18/104 and 26%, 23/88 at exposure to "purified" and "commercial-grade" 2,3,4,6-T4CP, respectively) was significantly increased at $p < 0.05$ compared with the control incidence of 8%, 14/173. For "commercial-grade" 2,3,4,6-T4CP, the incidence of this skeletal variation was also significantly increased among litters (50%, 8/16) compared with the control incidence of 19%, 6/31. For "purified" 2,3,4,6-T4CP, the incidence among litters (35%, 7/20) was not significantly different from the control incidence. This variation occurs normally in control populations of this strain of rats. Therefore, the increased incidence of this variation is considered to be a nonspecific effect (indicative of delayed development), not a teratogenic effect. At 10 $mg.kg^{-1}$ $bw.day^{-1}$, a significantly increased incidence of subcutaneous edema was found at exposure to either compound. This soft tissue variation, also observed among control animals, was not observed at 30 $mg.kg^{-1}$ $bw.day^{-1}$ of either compound and, therefore, considered to be not treatment-related (Schwetz et al., 1974a). *In a similar teratology study with "purified" PCP (see below, Schwetz et al., 1974b), the incidence among litters of this skeletal variation was significantly increased at 5 mg PCP.kg^{-1} bw.day^{-1} (60% versus 19% among control litters). The dose level of 30 mg.kg^{-1} bw.day^{-1} of "commercial-grade" 2,3,4,6-T4CP is equivalent to 8 mg PCP.kg^{-1} bw.day^{-1}*. Therefore, the slightly higher incidences of this skeletal variation at exposure to "commercial-grade" 2,3,4,6-T4CP compared to that at exposure to "purified" 2,3,4,6-T4CP can be explained by the PCP content.

PCP

A reproduction study in which female rats were exposed to "technical-grade" PCP (purity 85%) in feed, from 3 weeks of age through gestation, resulted in a decreased litter size at 500 $mg.kg^{-1}$ feed (equivalent to 25 $mg.kg^{-1}$ $bw.day^{-1}$); a concentration of 50 $mg.kg^{-1}$ feed (equivalent to 2.5 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect on reproductive performance. Maternal toxicity was not observed at any concentration tested. An extension of the exposure of the dams through lactation, followed by exposure of the progeny for an

additional 10 weeks, resulted in effects on the progeny: pre-and postnatal exposure resulted in a decreased DTH-response and a decreased serum BSA antibody concentration at all dose levels tested (5, 50 and 500 $mg.kg^{-1}$ feed, equivalent to 0.25, 2.5 and 25 $mg.kg^{-1}$ $bw.day^{-1}$). Therefore, a dose without effect can not be derived from this study. Additionally, the number and phagocytic activity of peritoneal macrophages were increased at 50 and 500 $mg.kg^{-1}$. A second part of this study is reported in section 1.2.3, "long-term exposure" (Exon and Koller, 1982, 1983a,b; table 1.3 and 1.4).
In a fertility and reproduction study, male and female Sprague-Dawley rats were exposed to Dowicide EC-7 (90% PCP) in feed. Females were exposed from 9 weeks prior to mating through gestation and lactation. Parent males were exposed for another two months. At 30 $mg.kg^{-1}$ $bw.day^{-1}$, body weight gain of adult females, the number of liveborn pups, neonatal survival and neonatal body weight were reduced. In addition, there was an increased number of litters which showed variations in the development of skeletal structures, namely lumbar spurs and variations of vertebrae. It is not reported whether these variations were found also in control animals in the present study or not, but the same variations occured in control animals in a previous teratology study (see Schwetz et al., 1974b). Therefore, the increased incidence of these variations is considered to be a nonspecific effect (indicative of delayed development), not a teratogenic effect. A dose level of 3 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect (Schwetz et al., 1978; table 1.3).
In another fertility and reproduction study, 5-w old male and female Sprague-Dawley rats were exposed to highly purified PCP (purity > 99%) in feed. Females were exposed through gestation. At 200 $mg.kg^{-1}$ feed (equal to 13 $mg.kg^{-1}$ $bw.day^{-1}$) the number of dams with ≥ 2 resorptions was increased, and foetal body weight was reduced. At this dose level, misshapen centra of wavy ribs was the only skeletal variation that was significantly increased; the incidence was 22 out of 86 *versus* 14 out of 167 in controls. The increased incidence of this skeletal variation is considered to be a nonspecific effect, not a teratogenic effect. At 600 $mg.kg^{-1}$ feed (equal to 43 $mg.kg^{-1}$ $bw.day^{-1}$) all but one foetuses were resorbed; at this dose level maternal weight gain during gestation was reduced, and ringed eye was observed in 50% of the dams. In this study PCP was found to be slightly more toxic with regard to maternal and reproductive effects than pentachloroanisole (PCA), a metabolite of PCP which can be formed by biological systems (Welsh et al., 1987; table 1.3).

In a teratology study ("segment II" study) the embryo-/foetotoxicity and teratogenicity of two grades of PCP were studied in rats. Groups of 20

Sprague-Dawley rats were treated by gavage with doses of 0 (vehicle control), 5, 15, 30 or 50 $mg.kg^{-1}$ $bw.day^{-1}$ (in corn oil) from day 6 through day 15 of gestation. The highest dose level tested was the highest dose without signs of toxicity in a preliminary 10-d tolerance study (table 1.2). At the 5 and 30 $mg.kg^{-1}$ $bw.day^{-1}$ dose levels, the amount of the commercial product was adjusted to provide 5 and 30 mg PCP $.kg^{-1}$ $bw.day^{-1}$, respectively. Animals were killed on day 21 of gestation. The two grades of this compound were "commercial-grade" PCP (purity 88%; 4% T4CP; 6% higher chlorinated phenoxyphenols; 30 ppm HxCDF, 80 ppm HpCDF, 80 ppm OCDF, 4 ppm HxCDD, 125 ppm HpCDD, 2,500 ppm OCDD, < 0.05 ppm 2,3,7,8-TCDD) and "purified" (purity ≥ 98% PCP; 0.3% T4CP; higher chlorinated phenoxyphenols 0.5%; < 1 ppm of HxCDF, HpCDF, OCDF, HxCDD, HpCDD and OCDD; < 0.05 ppm 2,3,7,8-TCDD).

At 30 and 50 $mg.kg^{-1}$ $bw.day^{-1}$, maternal weight gain on days 6 through 21 was significantly ($p < 0.05$) reduced, regardless of the compound tested. No other signs of maternal toxicity were observed with any dose of either compound. Treatment with "commercial-grade" PCP at ≥ 15 $mg.kg^{-1}$ $bw.day^{-1}$ resulted in one or more of the following reproductive effects: increased number of foetal resorptions, reduced foetal body weight and/or altered sex ratio toward male animals; resorption was the most sensitive parameter. Treatment with "purified" PCP at ≥ 30 $mg.kg^{-1}$ $bw.day^{-1}$ also affected reproductive performance.

Treatment with either compound resulted in dose-related increased incidences of skeletal variations of the skull (delayed ossification), vertebrae and sternabrae, and of subcutaneous edema; these variations were also observed in the vehicle controls. The only treatment-related variations which were not observed in control foetuses, were rib anomalies (supernumerary, lumbar or fused). These anomalies, and the increased incidences of skeletal and soft tissue variations are considered to be nonspecific (embryo-/foetoxic) effects, not teratogenic effects. In the study with "purified" PCP, the incidence of one of these variations, delayed ossification of skull bones, was already significantly increased at 5 $mg.kg^{-1}$ $bw.day^{-1}$ at this dose level (incidence among exposed litters 60%, 9/15 *versus* 19%, 6/31 in control litters). In the study with "commercial-grade" PCP, the dose of 5 $mg.kg^{-1}$ $bw.day^{-1}$ resulted in a two-fold increase in the incidence of both delayed ossification of skull bones, and of lumbar spurs, but these increases were statistically not significant at the $p \leq 0.05$ level. Additional studies in which groups of pregnant rats were given 0 or 30 $mg.kg^{-1}$ $bw.day^{-1}$ of either compound on days 8-11 or 12-15 of gestation showed that maternal and foetal body weights, foetal

resorptions, and a number of foetal variations were much more affected during early organogenesis than during late organogenesis. Treatment during early organogenesis was about as effective as treatment during the whole period of organogenesis (Schwetz et al., 1974b).

The embryo-/foetotoxicity and teratogenicity of PCP was also investigated in two oral studies with Charles River CD rats. In the one study, pregnant rats were exposed by intubation from days 7 through day 18 of gestation to 0 (vehicle control) or 75 mg PCP.kg^{-1} bw.day^{-1} (purity not reported; in corn oil). The animals (6 control and 7 treated animals, respectively) were sacrificed 1-2 days before parturition. Treatment resulted in significantly decreased foetal body weights. The average number of abnormal foetusses per litter was 0.7 *versus* 0 in controls and maternal weight gain was reduced 45%; these differences are statistically not significant. foetal mortality and the number of viable foetusses/litter were not affected either (Courtney et al., 1976). In the other study, pregnant rats were orally (no further details reported) exposed to a single dose of 0 (vehicle control) or 60 mg PCP.kg^{-1} bw (purity > 99%; in olive oil) on one of the following days of gestation: 8, 9, 10, 11, 12 or 13. The animals (5 control animals and 6 treated animals per group) were sacrificed on day 20 of gestation. Treatment on day 8 and on day 9 resulted in one (1%) abnormal foetus (dwarf) and three (6%) abnormal foetusses (exencephaly, macropthalmia, taillessness), respectively. Treatment on day 9 and on day 10 resulted in significant ($p < 0.05$) reduced foetal body weights. These effects may be due to maternal toxicity, as indicated by an increase in body temperature measured in animals exposed on day 8, 9 or 10. The percentage of resorptions in control (2%-12%) and treated groups (2%-13%) was similar (Larsen et al., 1975).

Oral administration of PCP (purity of test compound and route of administration not reported) to pregnant hamsters at doses of 1.25 to 20 mg.kg^{-1} bw.day^{-1} on days 5-10 of gestation, resulted in resorptions and foetal deaths in 3 of 6 test groups. The four intermediate dose levels or other data are not available (Hinkle, 1973, abstract).

Additional data

Intraperitoneal injection of mice with either 50-100 mg 2,4,6-T3CP.kg^{-1} bw (purity 99%) or 50-100 mg PCP.kg^{-1} bw (purity 99%) on day 10 of gestation resulted in loss of litters, reduced litter sizes and increased mortality

in the progeny. Data on maternal toxicity are not reported (Fahrig et al., 1978).

The mouse *in vitro* fertilization assay, in which both ova and sperm are exposed to the test compound, was used as a preliminary screening method for reproductive toxicity of dichlorophenols. The compounds 2,5-DCP, 3,4-DCP and 3,5-DCP significantly reduced sperm penetration of ova at a concentration of 1 mM; the remaining dichlorophenols were without effect. In an additional experiment, neither *in vitro* sperm penetration of control ova nor sperm motility was affected after a 90-d exposure of male mice to 2,4-DCP, at concentrations up to 500 $mg.l^{-1}$ drinking water (Seyler et al., 1984).

Summary and conclusions "reproductive toxicity"

Teratology studies

The embryo-/foetotoxicity and teratogenicity of 2,4-DCP (purity 99%), two different grades of 2,3,4,6-T4CP ("purified", purity > 99% and "commercial-grade", purity 73%) and of two different grades of PCP ("purified", purity ≥ 98% and "commercial-grade", purity 88%) have been investigated in oral studies with rats, in accordance with the protocol for so-called "segment II" studies. On the basis of these studies it is concluded that there is no evidence for teratogenicity of these compounds. However, 2,4-DCP may be embryo-/foetotoxic (the effects observed were associated with maternal toxicity), and 2,3,4,6-T4CP and PCP are embryo-/foetotoxic at concentrations at which maternal toxicity is not evident.

Doses of 375 $mg.kg^{-1}$ $bw.day^{-1}$ of 2,4-DCP, 10 $mg.kg^{-1}$ $bw.day^{-1}$ of both "purified" and "commercial"-grade 2,3,4,6,-T4CP and 5 $mg.kg^{-1}$ $bw.day^{-1}$ of "commercial-grade" PCP were without effect with regard to both developmental effects (non-specific effects such as delayed ossification) and reproductive effects (such as reduced litter size and reduced foetal body weight). In the study with "purified" PCP, the lowest dose level tested (5 $mg.kg^{-1}$ $bw.day^{-1}$) resulted in an increased incidence of delayed ossification of skull bones.

The rat studies with 2,3,4,6-T4CP and PCP indicate that the non-phenolic impurities (polychlorinated dibenzo-p-dioxins and dibenzofurans) present in the "commercial-grade" compounds do not contribute significantly to the effects of these compounds on reproductive and developmental effects, neither qualitative nor quantitative.

Additional teratology studies with PCP also showed no evidence for teratogenicity; embryo-/foetotoxic effects observed in these studies appeared to be associated with maternal toxicity.

Reproduction studies

The effects of 2-MCP, 2,4-DCP, 2,4,6-T3CP and PCP on reproductive performance (judged by parameters such as fertility, litter size, number of stillborn, and birth weight) have been investigated in a number of oral studies. In most of these studies, female rats were exposed from weaning age through gestation. In some of these studies, exposure of the dams was continued through lactation and followed by exposure of the progeny for an additional 10-15 weeks, to study effects on the progeny exposed both pre- and postnatally. On the basis of these studies it is concluded that 2-MCP, 2,4-DCP, 2,4,6-T3CP and PCP are embryo-/foetotoxic at concentrations at which maternal toxicity is not evident (This conclusion is consistent with the results observed in the teratology studies). Based on these studies the following NO(A)ELs have been derived with respect to embryo-/foetotoxicity: 5 $mg.kg^{-1}$ $bw.day^{-1}$ for 2-MCP, 3 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4-DCP, 3 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4,6-T3CP, and 2.5 to 4 $mg.kg^{-1}$ $bw.day^{-1}$ for different PCP-formulations ("highly-purified" PCP, "Dowicide EC-7" and "technical-grade" PCP). These data (and the lowest effect-levels) show that the embryo-/ foetotoxicity of the chlorophenols studied is very similar. The studies with the different grades of PCP show that the non-phenolic impurities in "Dowicide EC-7" ("low-impurity" grade) and in "technical-grade" PCP ("high-impurity" grade) do not contribute significantly to the effects of these compounds with regard to embryo-/ foetotoxicity, consistent with the results of the teratology studies.

The studies in which the progeny was exposed both pre- and postnatally show that exposure to 2,4-DCP, 2,4,6-T3CP and "technical-grade" PCP results in effects on the progeny at dose levels that are lower than those affecting reproductive performance. In these studies, the immunocompetence (2,4-DCP, PCP) and liver and/or spleen weights (2,4-DCP, 2,4,6-T3CP, PCP) of the progeny -exposed both pre- and postnatally- were found to be sensitive parameters, resulting in NO(A)ELs of 0.3 $mg.kg^{-1}$ $bw.day^{-1}$ for both 2,4-DCP and 2,4,6-T3CP. The study with "technical-grade" PCP resulted in effects on the immunocompetence at all dose levels tested ($\geq$0.25 $mg.kg^{-1}$ $bw.day^{-1}$). These effects on the progeny have not been investigated in the studies with "highly-purified" PCP and "Dowicide EC-7".

1.2.3 Long-term exposure (semichronic and chronic toxicity) - noncarcinogenic and carcinogenic effects

Animal data

Oral exposure

The results of long-term, oral studies are summarized in table 1.4. If more than one study was available for one compound, the studies are listed in the following order: i) animal species (from "small" to "large"), ii) exposure time (from "short" to "long"), and iii) purity of test compound (from "high purity" to "low purity"). For each study, a lowest-effect-dose, LED, and a no-observed-(adverse)-effect-level, NO(A)EL, are listed in the table, if possible. These values are based on an evaluation of the data reported and do not necessarily represent the opinion of the investigators. Effects on survival, body weight, weight of major organs, gross pathology and histopathology are common endpoints studied in most of these long-term studies. In a number of studies other endpoinds such as carcinogenicity, haematology and clinical chemistry, were studied as well.
In the text below, especially the effects observed at the lowest-effect-dose (LED) are discussed. For data on the effects observed at higher dose levels the reader is referred to table 1.4.
Dose levels are expressed as $mg.kg^{-1}$ $bw.day^{-1}$, regardless of treatment procedure.

2-MCP

The effects of 2-MCP (purity 97%), in drinking water, has been investigated in a 2-yr two-generation study with Sprague-Dawley rats. Animals of the first generation (females only) were exposed from 3 weeks of age through gestation and lactation, to study effects on reproductive performance. Animals of the second generation (exposed prenatally) were continued on treatment untill tumour development, death or termination at 24 months, to study carcinogenicity and toxicity after pre- and postnatal exposure. Exposure to dose levels up to 50 $mg.kg^{-1}$ $bw.day^{-1}$ did not result in an effect on tumour incidence, latency or type. In the second year of the study, red blood cell count, packed cell volume and haemoglobin content were increased at this dose level. The effects on reproductive performance of the first generation and the effects on the progeny during the first 6 months of the study are discussed in section 1.2.2. Considering all

parameters studied, a dose level of 5 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect. In parallel studies the influence of 2-MCP on ethylnitrosourea (ENU)-induced tumour formation was studied, by "simultaneous" exposure to ENU and 2-MCP. ENU was given to the first generation as the precursors, 0.32% ethylurea in feed and 1 mg $NO_2.l^{-1}$ in drinking water, during gestation days 14 to 21. The second generation, in which tumour formation was studied, was exposed to 2-MCP (as described above) either prenatally only, postnatally only (from weaning though the remaining part of the study) or pre- and postnatally. In these studies, the tumour incidence in male offspring was generally increased and the tumour latency was decreased at all dose levels of 2-MCP, compared with ENU-only treated controls (these parameters were calculated at three time intervals corresponding to 25%, 50% and 75% of combined tumour incidence in males and females exposed to ENU only). However, the differences were mostly not statistically significant at $p \leq 0.10$ and not dose-related. In female offspring tumour incidence and latency were not consistently affected by simultaneous exposure, compared with ENU-only treated controls (Exon and Koller, 1982, 1983a,b, 1985; table 1.3 and 1.4).

2,4-DCP

Mice

Exposure of mice for 3 months to 2,4-DCP (purity > 99%) in feed did not result in an effect on body weight gain at 1,400 $mg.kg^{-1}$ $bw.day^{-1}$, although feed consumption was reduced > 20%. Therefore, 1,400 $mg.kg^{-1}$ $bw.day^{-1}$ is considered to be an effect-dose. At 700 $mg.kg^{-1}$ $bw.day^{-1}$, both weight gain and feed consumption were similar to controls; this dose is considered to be the dose without (adverse) effect, although 4 of 10 males exposed to this dose level showed hepatocellular necrosis, the severity of which was judged to be "minimal", level (NTP, 1989a). Exposure of mice for 3 months to 2,4-DCP (purity > 99%) in drinking water at dose levels up to 385 $mg.kg^{-1}$ $bw.day^{-1}$ (males) and 490 $mg.kg^{-1}$ $bw.day^{-1}$ (females) did not result in toxicologically significant alterations (Borzelleca et al., 1985b).
In a study in which male mice were exposed for 6 months to 2,4-DCP (purity not reported) in feed, minor histological changes (infiltration of round cells; swelling of hepatocytes) were observed in the liver of 1 or 2 out of 7 animals at 230 $mg.kg^{-1}$ $bw.day^{-1}$; a dose of 100 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect (Kobayashi et al., 1972).

A 2-year carcinogenicity and toxicity study in which B6C3F1 mice were exposed to 2,4-DCP (purity > 99%) in feed, at dose levels of 0, 800 and 1,300 $mg.kg^{-1}$ $bw.day^{-1}$ (males) or 0, 430 and 820 $mg.kg^{-1}$ $bw.day^{-1}$ (females), did not result in compound-related increases in malignant or benign neoplasms, neither in males nor in females. At 820 $mg.kg^{-1}$ $bw.day^{-1}$, body weight of females was reduced progressively throughout the study; a dose of 430 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect. In males a dose-related increased incidence of diffuse syncitial alteration of hepatocytes was observed (11/50 in controls; 33/49 and 42/48 at low and high dose, respectively); the increase was statistically significant at both concentrations tested. Therefore, a dose without effect for males could not be established (NTP, 1989a).

Rats

Exposure of rats for 3 months to 2,4-DCP (purity > 99%) in feed, resulted in lower body weights of both sexes, at 1,000 $mg.kg^{-1}$ $bw.day^{-1}$. In addition, bone marrow atrophy was observed in all animals exposed to this dose level and in 6 of 10 females at 500 $mg.kg^{-1}$ $bw.day^{-1}$. Dose levels of 500 $mg.kg^{-1}$ $bw.day^{-1}$ (males) and 250 $mg.kg^{-1}$ $bw.day^{-1}$ (females) were without effect (NTP, 1989a).

A 2-year carcinogenicity and toxicity study in which F344/N rats were exposed to 2,4-DCP (purity > 99%) in feed, at dose levels of 0, 210 and 440 $mg.kg^{-1}$ $bw.day^{-1}$ (males) or 0, 120 and 250 $mg.kg^{-1}$ $bw.day^{-1}$, did not result in compound-related increases in malignant or benign neoplasms, neither in males nor in females. Body weight of males was reduced at 440 $mg.kg^{-1}$ $bw.day^{-1}$. Furthermore, a dose-related increased incidence of multifocal degeneration of respiratory epithelium of the nose was observed in males (25/45 in controls; 38/48 and 42/46 at low and high dose, respectively). Although the increase of this incidence was statistically significant at both dose levels, the dose of 210 $mg.kg^{-1}$ $bw.day^{-1}$ is considered to be the dose without (adverse) effect in males. Body weight of females was reduced at 250 $mg.kg^{-1}$ $bw.day^{-1}$; the dose of 120 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect (NTP, 1989a).

The effects of 2,4-DCP (purity 99%), in drinking water, has been investigated in a 2-yr two-generation study with Sprague-Dawley rats. Animals of the first generation (females only) were exposed from 3 weeks of age through gestation and lactation, to study effects on reproductive performance. Animals of the second generation (exposed prenatally) were

continued on treatment untill tumour development, death or termination at 24 months, to study carcinogenicity and toxicity after pre- and postnatal exposure. Exposure to dose levels up to 30 $mg.kg^{-1}$ $bw.day^{-1}$ did not result in an effect on tumour incidence, latency or type. In the second year of the study, red blood cell count and haemoglobin content were increased at this dose level. The effects on reproductive performance of the first generation and the effects on the progeny during the first 6 months of the study are discussed in section 1.2.2. Considering all parameters studied, a dose level of 0.3 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect. In parallel studies the influence of 2,4-DCP on ethylnitrosourea (ENU)-induced tumour formation was studied, by "simultaneous" exposure to ENU and 2-MCP. ENU was given to the first generation as the precursors, 0.15% ethylurea in feed and 1 mg $NO_2.l^{-1}$ in drinking water, during gestation days 14 to 21. The second generation, in which tumour formation was studied, was exposed to 2,4-DCP (as described above) either prenatally only, postnatally only (from weaning though the remaining part of the study) or pre- and postnatally. In these studies, the tumour incidence and latency, determined at termination, were not affected by simultaneous exposure, compared to the ENU-only treated controls. However, in the ENU-only treated controls the tumour incidence was not increased compared to that in untreated controls, because of the relatively low ethylurea level in feed (Exon et al., 1984; Exon and Koller, 1985; table 1.3 and 1.4).

2,4,5-T3CP

Exposure of rats for 3 months to 2,4,5-T3CP (purity > 99%) in feed, resulted in pathological changes in kidneys ("moderate degenerative changes in the epithelium lining of the convoluted tubules and early proliferation of the interstitial tissue") and in liver ("mild centrolobular degenerative changes characterized by cloudy swelling and an occasional area of focal necrosis"), at dose levels of 150 and 500 $mg.kg^{-1}$ $bw.day^{-1}$. The severity of these lesions was reported to be dose-related. In addition, a diuretic effect was observed at these dose levels. A dose of 50 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect (McCollister et al., 1961). It must be noted that the histopathological data have been reported very briefly.

2,4,6-T3CP

The carcinogenicity and toxicity of 2,4,6-T3CP (purity 96%-97%) has been investigated in 2-year feed studies with B6C3F1 mice and F344/N rats (NCI, 1979).

In the one study, male mice were exposed to dose levels of 0, 700 and 1,400 $mg.kg^{-1}$ $bw.day^{-1}$ and female mice were exposed to dose levels of 0, 750 and 1,500 $mg.kg^{-1}$ $bw.day^{-1}$. With regard to carcinogenicity, dose-related increased incidences of hepatocellular carcinomas and hepatocellular adenomas were observed in males and in females. In males the incidence of both carcinomas and adenomas was significantly increased at both dose levels; in females only the increased incidence of adenomas at the high-dose level was statistically significant. These types of hepatocellular neoplasms normally occur in control populations of this strain of mice, especially in males. In addition to neoplasms, body weights were dose-related decreased throughout the study, and non-neoplastic hepatocellular lesions were commonly present in dosed animals.

In the other study, male and female rats were exposed to dose levels of 0, 250 and 500 $mg.kg^{-1}$ $bw.day^{-1}$. With regard to carcinogenicity, a dose-related increase in leukemias was observed in males; the incidence was significantly increased at both dose levels. In females there also was an increased incidence of leukemias, but the incidence was not statistically significant at any dose level. Leukemias normally occur in control populations of this strain of rats. In addition to neoplasms, body weights were dose-related decreased throughout the study. The incidences of non-neoplastic lesions were within normal limits in all groups (NCI, 1979).

In a preliminary oral carcinogenicity study using two F1 hybrid stocks of mice, (C57BL/6 x C3H/Anf)F1 and C57BL/6 x AKR)F1, 18 1-w old animals of each sex per group were exposed by stomach tube to a dose level of 100 mg "Omal".kg^{-1} $bw.day^{-1}$ for 3 weeks and subsequently to a dose level of 260 mg "Omal".kg^{-1} in feed (equivalent to 40 $mg.kg^{-1}$ $bw.day^{-1}$) for 18 months. Treatment resulted in "an elevation of tumour incidence in an uncertain range, which require additional evaluation" (Innes et al., 1969). Additional data on this study have not been reported. Therefore, this study has not been summarized in table 1.4.

PCP

Mice

The toxicity of 2 different grades of PCP, namely "pure" PCP (purity > 99%) and "technical-grade" PCP (purity 86%), has been investigated in 3-mo comparative feed studies, at dose levels of 0, 7 and 70 $mg.kg^{-1}$ $bw.day^{-1}$ (The impurities in these formulations were not reported). Histopathological examinations showed similar, dose-related liver lesions, regardless of test compound: mild to marked swelling of hepatocytes, accompanied by nuclear swelling and vacuolization, and eosinophilic inclusion bodies within nuclear vacuoles; mild to moderate necrosis was observed only at 70 $mg.kg^{-1}$ $bw.day^{-1}$. However, exposure to "technical-grade" PCP resulted in dose-related enhancements of immunologically mediated susceptability to tumour induction after a challence in "host susceptibility models", and further in a decreased T-cell cytolytic activity and increased phagocytic activity of macrophages, while exposure to "pure" PCP did not affect these immunological parameters. These data indicate that the effects on the immunocompetence is associated with the contaminant(s) present in "technical-grade" PCP. However, in surviving "pure" PCP exposed animals that were resistent to both the MSV and MSB challenge (see table 1.4 for explanation), a dose-related increase in gross tumours in spleen (2/9 and 4/9 *versus* 0/13 in control animals) was observed; this suggests some degree of immunosuppression by PCP itself (Kerkvliet et al., 1982).

The toxicity of 4 different grades of PCP has been investigated in 6-mo comparative feed studies with B6C3F1 mice. The lowest and highest dose tested in each study were 28 and 170-255 $mg.kg^{-1}$ $bw.day^{-1}$, respectively. Test compounds were i] "pure" PCP (purity 98.6%), ii] "Dowicide-EC-7" (91% PCP), iii] "DP-2" (92% PCP), and iv] "technical-grade" PCP (90% PCP). The main impurities in these compounds are i] T4CP and chlorohydroxy-dibenzofurans and -diphenyl ethers, ii] T4CP, iii] T4CP, chlorohydroxy-diphenyl ethers and -dibenzofurans, PCDF and PCDD, and iv] T4CP, chlorohydroxy-diphenyl ethers, and -dibenzofurans, PCDF and PCDD, respectively.

In all 4 studies, compound-related histopathological changes were found in several tissues, especially in the liver. Liver weights were increased at all dose levels, although there were some quantitative differences. Additionally, most animals examined histologically showed similar liver changes, namely necrosis, nuclear alteration, cytomegaly and pigmentation,

regardless of compound tested and dose level. Bile duct hyperplasia was observed in all animals exposed to 255 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP; this lesion was not or scarcely observed in animals exposed to the other compounds (highest dose levels: 170-210 $mg.kg^{-1}$ $bw.day^{-1}$). Exposure to any compound did not result in hepatic porphyria. Other tissues affected were galbladder, bone marrow, spleen, thymus, testes, urinary bladder and nasal mucosa. Spleen weights of males were increased in all studies, while those of females were decreased in high-dosed groups (except at exposure to "pure" PCP).

A marked difference between the different compounds was observed with respect to the induction of cytochrome P450-mediated aryl hydrocarbon hydroxylase (AHH) in liver microsomes. Exposure to "technical-grade" PCP and "DP-2" resulted in a 30-fold increase in AHH activity at dose levels of 28 and 85 $mg.kg^{-1}$ $bw.day^{-1}$, respectively. Exposure to "pure" PCP and "Dowicide EC-7" only resulted in a 5-fold increase in AHH activity at dose levels of 210 and 170 $mg.kg^{-1}$ $bw.day^{-1}$, respectively. The ability of "technical-grade" PCP and "DP-2" to induce AHH is consistent with their relatively high content of PCDF and PCDD, which are known inductors of AHH. Based on the limited ability of "pure" PCP and "Dowicide EC-7" to induce the AHH activity, it can not be excluded that PCP itself caused this effect at the high dose levels tested (210 and 170 $mg.kg^{-1}$ $bw.day^{-1}$, respectively). A further difference between the compounds was observed with regard to an immunological parameter: the plague-forming cell (PFC) response following immunization with sheep erythrocytes was markedly inhibited at exposure to "technical-grade" PCP and (to a lesser degree) at exposure to "DP-2", while this antibody response was not suppressed by the other two compounds.

These studies show that the effects of the 4 different grades of PCP are similar with respect to most parameters studied, although quantitative differences have been observed with regard to a number of these parameters. However, the induction of AHH and the suppression of the PFC response observed in the studies with "technical-grade" PCP and "DP-2" is (largely) consistent with the presence of impurities in these compounds (NTP, 1989b).

The carcinogenicity and toxicity of two different grades of PCP have been investigated in 2-yr comparative feed studies with B6C3F1 mice. Test compounds were "Dowicide EC-7" (91% PCP; "low" content of PCDF and PCDD) and "technical-grade" PCP (90% PCP; "high" content of PCDF and PCDD). Dose levels were 0, 17, 35 and 116 $mg.kg^{-1}$ $bw.day^{-1}$ of "Dowicide EC-7" and 0, 17 and 35 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP.

In the study with "Dowicide EC-7", dose-related increased incidences of both malignant and benign neoplasms were observed in both sexes. In males the incidences of hepatocellular carcinomas, hepatocellular adenomas and benign adrenal medullary pheochromocytomas were increased (statistically significant at 35 and/or 116 $mg.kg^{-1}$ $bw.day^{-1}$). In females the incidences of hepatocellular adenomas, benign adrenal medullary pheochromocytomas and hemangiosarcomas in spleen and liver were increased (statistically significant at 116 $mg.kg^{-1}$ $bw.day^{-1}$). The types of neoplasms observed in this study normally occur in control populations of this strain of mice, especially hepatocellular carcinomas and adenomas. In addition to neoplasms, high to very high incidences of histopathological liver changes (acute diffuse necrosis, diffuse chronic active inflammation, diffuse cytomegaly, and multifocal pigmentation were observed in all dosed groups, but not in control groups. Further, body weight of females was reduced at 116 $mg.kg^{-1}$ $bw.day^{-1}$, and a very high incidence of bile duct hyperplasia was observed at this dose level in both sexes.

The study with "technical-grade" PCP resulted in dose-related increased incidences of both malignant and benign neoplasms in males and of malignant neoplasms in females. In males the incidences of hepatocellular carcinomas, hepatocellular adenomas and benign adrenal medullary pheochromocytomas were increased (statistically significant at 17 and 35 $mg.kg^{-1}$ $bw.day^{-1}$). In females the incidence of hemangiosarcomas in spleen and liver was increased (statistically significant at 35 $mg.kg^{-1}$ $bw.day^{-1}$ only). In addition to neoplasms, a very high incidence of histopathological liver changes (acute diffuse necrosis, diffuse chronic active inflammation, diffuse cytomegaly, and multifocal pigmentation were observed in all dosed groups, but not in control groups. Further, a high incidence of bile duct hyperplasia was observed in dosed males, but not in dosed females (NTP, 1989b).

In the aforementioned NTP-report, a comparison has been made between the incidences of hepatocellular neoplasms (adenomas plus carcinomas) observed in male B6C3F1 mice in the carcinogenicity studies with "Dowicide EC-7" and "technical-PCP" and those observed in male mice in carcinogenicity studies with either HxCDD (a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD) and 2,3,7,8-TCDD. This comparison showes that HxCDD alone accounts for only a small part of the hepatocellular neoplasms observed in the studies with "Dowicide EC-7" and "technical-grade" PCP. Therefore, and because of the very low content of impurities in "Dowicide EC-7", the data indicate that PCP itself must be considered to be carcinogenic in this strain of mice. Additionally, (increased incidences of) adrenal medullary pheochromocytomas

and of hemangiosarcomas were not observed in the studies with HxCDD or 2,3,7,8-TCDD (NTP, 1989b).
In a preliminary oral carcinogenicity study using two F1 hybrid stocks of mice, (C57BL/6 x C3H/Anf)F1 and C57BL/6 x AKR)F1, 18 1-w old animals of each sex per group were exposed by stomach tube to a dose level of 46 mg "Dowicide-7".kg^{-1} bw.day^{-1} for 3 weeks and subsequently to a dose level of 130 mg "Dowicide".kg^{-1} in feed (equivalent to 20 mg.kg^{-1} bw.day^{-1}) for 18 months. Treatment "did not result in a significant increase in tumours" (Innes et al., 1969). Additional data on this study have not been reported. Therefore, this study has not been summarized in table 1.4.

Rats

The toxicity of 2 different grades of PCP ("analytical-grade" and "technical-grade") was investigated in 3-mo comparative feed studies with rats, at dose levels 0, 3, 10 and 30 mg.kg^{-1} bw.day^{-1}. Exposure to "analytical-grade" PCP did not result in toxic effects at dose levels up to 30 mg.kg^{-1} bw.day^{-1}, while exposure to "technical-grade" PCP at a dose level of 30 mg.kg^{-1} bw.day^{-1} resulted in increased liver and kidneys weights, mild focal degeneration and necrosis in the liver, and increased haemoglobin, packed cell volume, red blood cell counts, serum alanine aminotransferase (ALAT) and alkaline phosphatase *(Kociba, 1971)*. In a follow-up study, *Kociba (1973)* exposed rats for 3 months to "analytical-grade" PCP at dose levels of 0, 1, 3, 10 or 30 mg.kg^{-1} bw.day^{-1}. From this study and from the studies by Schwetz et al. (1978; table 1.3 and table 1.4) with "Dowicide EC-7" it was determined by this group of investigators, that dose levels of 3 and 10 mg.kg^{-1} bw.day^{-1} of "analytical-grade" PCP are without effect for females and males, respectively (Exon, 1984b, secundary source; the Kociba studies are not available and therefore not summarized in table 1.4).

The toxicity of 3 differents grades of PCP, namely "technical-grade" PCP, "improved" PCP and "pure" PCP has been investigated in 3-mo comparative feed studies with rats, at dose levels of 0, 3, 10 and 30 mg.kg^{-1} bw.day^{-1}. These grades represent "high"-, "medium"- and "low"-impurity grades of PCP, respectively, with regard to the presence of PCDD (and other impurities). In the studies with either "pure" PCP or "improved" PCP, terminal weights of liver and kidneys were increased at 30 mg.kg^{-1} bw.day^{-1}; that of liver was also increased at 10 mg.kg^{-1} bw.day^{-1}. Histopathological lesions or other effects were not observed; the dose of 3 mg.kg^{-1} bw.day^{-1} was without

effect in these two studies. In the study with "technical-grade" PCP, terminal weights of liver and kidneys, and serum alkaline phosphatase were increased at all dose levels tested. Additionally, serum albumin was decreased at 10 and 30 $mg.kg^{-1}$ $bw.day^{-1}$, and histopathological liver changes (minimal focal hepatocellular degeneration and necrosis) were observed and haematological parameters (erythrocyte count, haemoglobin content, packed cell volume) were decreased at 30 $mg.kg^{-1}$ $bw.day^{-1}$ (Johnson et al., 1973).

The toxicity of another grade of PCP has been investigated in a 3-mo feed study with rats, at dose levels of 0, 1.25, 2.5, and 10 $mg.kg^{-1}$ $bw.day^{-1}$. In this study the following effects were observed at both 2.5 and 10 $mg.kg^{-1}$ $bw.day^{-1}$: increased liver weight (females), increased incidences of histopathological changes (centrilobular vacuolisation in the liver of males and a lower number of calculi in corticomedullary junction of the kidneys in females), and increased haematological parameters in males (number of erythocytes and haemoglobin content). Additionally, body weight gain of females was decreased, and serum alkaline phosphatase activity (females), serum glucose (males) and the activity of the microsomal enzymes aniline hydroxylase and aminopyrine demethylase was increased at 10 $mg.kg^{-1}$ $bw.day^{-1}$ (Knudsen et al., 1974). The PCP formulation tested by Knudsen et al. (1974) contained 200 ppm OCDD and is therefore considered to be a "medium"-impurity grade of PCP; data on other impurities were not reported.

The toxicity of 2 different grades of PCP has been investigated in 8-mo comparative feed studies with Sherman rats. Test compounds were "purified" PCP (purity > 99%; "very low" content of PCDF and PCDD) and "technical-grade" PCP (85% PCP, "high" content of PCDF and PCDD). Two studies were conducted with each compound, at dose levels of 0, 1, 5 and 25 $mg.kg^{-1}$ $bw.day^{-1}$. In addition to lethality and body weight, the one study was focused on organ weights and histopathology of major organs, while the other study was focused on hepatic enzymes.

In the studies with "purified" PCP, effects were only observed at 25 $mg.kg^{-1}$ $bw.day^{-1}$. At this dose level, body weights were reduced and several livers of females were totally dark or contained dark areas. Microscopic examinations showed minor hepatocellular alterations (slightly brownish diffuse discolouration in females, slightly enlarged hepatocytes around central veins in both sexes, cytoplasmatic eosinophilic inclusions in males and a brown pigment in macrophages of females). With regard to hepatic enzymes, a 3-fold increase in activity of glucuronyl transferase was measured; an effect on other enzymes was not observed. Indications of

hepatic porphyria were not found. The dose of 5 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect in both studies with this compound. In the studies with "technical-grade" PCP, hepatocellular alterations and effects on hepatic enzymes were observed at all dose levels tested. At the lowest dose (1 $mg.kg^{-1}$ $bw.day^{-1}$), centrolobular hepatocytes were slightly enlarged and occasionally vacuolated in all males and one female. With regard to hepatic enzymes, activities of aryl hydrocarbon hydroxylase and glucuronyl transferase were increased 3- and 15-fold, respectively, at 1 $mg.kg^{-1}$ $bw.day^{-1}$. Additionally, an altered ratio of the 455/430 nm peaks of the ethylisocyanide difference spectrum of cytochrome P450 was observed, caused by a shift from 455 to 453 nm. Exposure to dose levels of 5 and 25 $mg.kg^{-1}$ $bw.day^{-1}$ resulted in increases in cytochrome P450 content, microsomal heme, and liver and urine porphyrins. At these dose levels, several livers of females were totally dark or contained dark areas; some of these livers were fluorescent, indicating porphyria. The effects on hepatic enzymes and the occurence of hepatic porphyria are consistent with the high content of PCDF and PCDD which similarly produce these effects (Goldstein et al., 1977; Kimbrough and Linder 1978).

A 2-yr carcinogenicity and toxicity study in which Sprague-Dawley rats were exposed to "Dowicide EC-7" (90% PCP; "low" content of PCDF and PCDD) in feed, at dose levels of 0, 1, 3, 10 and 30 $mg.kg^{-1}$ $bw.day^{-1}$, did not result in compound-related increases in malignant or benign neoplasms. It must be noted that the number of animals used in this study was relatively low (27 of each sex/group) and that males were terminated after 22 months due to high mortality in all male groups. At dose levels of 10 and especially 30 $mg.kg^{-1}$ $bw.day^{-1}$, females showed dark discoloured livers and kidneys, caused by pigmentation. At 30 $mg.kg^{-1}$ $bw.day^{-1}$, body weight of females was reduced throughout the study and the activity of serum glutamic transaminase was increased in both sexes. Doses of 10 and 3 $mg.kg^{-1}$ $bw.day^{-1}$ were without effect in males and females, respectively (Schwetz et al., 1978).

Another 2-yr carcinogenicity and toxicity study with Sprague-Dawley rats also did not indicate a carcinogenic action of PCP, at exposure to dose levels up to 500 $mg.kg^{-1}$ feed (equivalent to 25 $mg.kg^{-1}$ $bw.day^{-1}$). In this 2-yr two-generation study, animals of the first generation (females only) were exposed from 3 weeks of age through gestation and lactation, to study effects on reproductive performance. Animals of the second generation (exposed prenatally, 24-28 animals of each sex per dose level) were continued on treatment untill tumour development, death or termination at

24 months, to study carcinogenicity and toxicity after pre- and postnatal exposure. The "technical-grade" PCP tested was a "medium-impurity" grade, based on the PCDD content (Exon and Koller 1983b; Exon, 1985, abstract). Further details on this study, with regard to carcinogenic and non-carcinogenic effects at long-term exposure, are not available; therefore this study is not summarized in table 1.4. Data on non-carcinogenic effects observed in the first 6 months of the study have been discussed in section 1.2.2, "reproductive toxicity" and summarized in table 1.3).

Exposure by inhalation

PCP

In inhalation studies in which rats and rabbits were exposed to an airborne PCP concentration of 3.0 $mg.m^{-3}$ for 4 hours per day, for 4 months, "minor" effects on liver function, cholinesterase activity and blood sugar were observed; these effects were no longer observed one month after completion of exposure. Exposure to 29 $mg.m^{-3}$ resulted in anaemia, leukocytosis, eosinophilia, hyperglycaemia and in dystrophic processes in the liver (Demidenko, 1969; in Russian; cited in WHO, 1987). [The number of experimental animals and the purity of the test compound is not reported] Exposure of weanling male rats to an airborne NaPCP ("reagent-grade") concentration of 21.4 $mg.m^{-3}$ for 4 hours per day, 6 days per week, for 4 months, significantly increased the weight of lungs, kidneys, liver and adrenal gland. In addition, blood glucose levels were increased throughout the study. These effects were not observed at 3.1 $mg.m^{-3}$. The number of experimental animals is not reported. In an identical study with rabbits (6 animals of each sex per group), liver weight was significantly increased at 3.1 $mg.m^{-3}$. In the high-dose group, liver and lung weights, and serum gamma-globulin were increased significantly (Ning et al., in Chinese; cited in WHO, 1987).

Chlorophenols other than PCP

Long-term animal data on chlorophenols other than PCP are not available.

Human data

This section on effects on humans at repeated exposure is based partly on the "Environmental Health Criteria Documents" on PCP (WHO, 1987) and on chlorophenols other than PCP (WHO, 1989).
There appear to be no studies or case reports on the effects of "pure" chlorophenols on humans. Therefore, the effects described in the present section may be influenced by the impurities present in the formulations used.
Most data on effects on humans at repeated exposure are available from occupational studies; exposure to chlorophenols is encountered in occupational settings such as chemical manufacturing (primarily DCP and T3CP) and the lumber industry (wood protection/preservation; primarily PCP, T4CP and T3CP). In occupational settings, it is difficult to distinguish between short-term and long-term exposure. Therefore, the data in the section below could not be separated on the basis of duration of exposure.

Occupational exposure - non-carcinogenic effects

PCP

Effects at repeated exposure include eye and skin irritation, irritation of mucous membranes and respiratory tract, signs of chloracne (ascribed to chlorinated impurities, in particular PCDD and PCDF), porphyria cutanea tarda, neurasthesia, depression, headaches, liver and kidney functional changes, and immunological changes. A number of these effects is observed already after short-term exposure, especially non-specific central nerve system effects. Little is known on airborne exposure levels at which these effects may occur. In many occupational settings, the effects observed are the result of both dermal exposure and exposure by inhalation. Effect levels also are obscured by mixed exposure to PCP and other chlorophenols and/or nonphenolic compounds (WHO, 1987).
Irritating effects have been reported at airborne PCP concentrations $\geq$ 1,000 $\mu g.m^{-3}$; workers accustomed to exposure may tolerate concentrations up to about 2,500 $\mu g.m^{-3}$ (WHO, 1987).
Workers exposed to "less than" 30 $\mu g.m^{-3}$ were reported to be in good health, but the prevalence of skin pustular eruptions was higher than expected. In a small group of workers (n = 8) exposed to an average concentration of 65 ($\pm$ 100) $\mu g.m^{-3}$ for 5 to 10 years, a correlation between

exposure level and serum and urine PCP concentrations was reported; this exposure level did not result in respiratory or dermatological effects. The workers were exposed to PCP (and T4CP) by inhalation only, in a lumber treatment plant (WHO, 1987). In a study among 22 "open vat wood treaters" (exposure to a 5% PCP solution in kerosene; exposure by inhalation and by direct contact with either the solution or the treated wood) and 24 "pressure tank wood treaters" (mixed exposure to PCP and other preservatives, for example chromium, arsenic and dieldrin), blood serum PCP levels due to long-term exposure were measured, resulting in values of 0.15-17.4 (mean 3.8) $mg.l^{-1}$ and 0.02-7.7 (mean 1.7) $mg.l^{-1}$, respectively. In the control group (n = 32) these values were 0.02-7.2 (mean 0.3) $mg.l^{-1}$). In a combined PCP exposure group consisting of 7 "open vat" and 10 "pressure tank" workers, strong to moderate statistical associations were observed between exposure to PCP and a number of clinical findings (increase in immature leucocytes, basophils, plasma cholinesterase, alkaline phosphatase, gamma-globulin and uric acid; decrease in serum calcium). However, most values were within their clinically normal range. These findings did not provide evidence for liver or other organ damage. An extensive medical examination of all PCP exposed workers (n = 46) and controls (n = 42) did not indicate serious health effects due to PCP exposure, although the standardized prevalence rates (SPR) for chronic sinusitis and chronic upper respiratory conditions were 3.4 and 2.8, respectively. The SPR for other illness conditions were below 2 which is considered to be insignificant (Klemmer et al., 1980). A comparison with a study reviewed by WHO (1987) showes that the average blood-serum levels of the lumber treatment workers (studied by Klemmer et al., 1980) were 7-16 times higher than those of workers exposed by inhalation to an average airborne PCP concentration of 65 $\mu g.m^{-3}$, and 2-5 times higher than those of workers exposed by inhalation to an average PCP concentration of 55 $\mu g.m^{-3}$; the workers exposed to 55 $\mu g.m^{-3}$ were also dermally exposed. This comparison showes that the airborne exposure level of the lumber treatment workers may well have exceeded 55-65 $\mu g\ PCP.m^{-3}$.

Haematological, neurological and skin effects have been reported among workers exposed to airborne levels of PCP and NaPCP between 30 and 1,000 $\mu g.m^{-3}$; 20% of the air samples collected in this study exceeded 200 $\mu g.m^{-3}$. The disorders reported may have been influenced by simultaneous exposure to hexachlorobenzene levels of 1,800 to 2,700 $\mu g.m^{-3}$, and by dermal exposure (WHO, 1987). According to Sterling et al. (1982), long-term exposure to chlorophenol wood preservatives (water soluble formulations containing sodium pentachlorophenate and/or tetrachlorophenate) at airborne

concentrations which were "well below" 50 $\mu g.m^{-3}$ has resulted in a slower elimination rate of the chlorophenols and in chronic effects such as respiratory disorders, persistent skin rashes and lesions, persistent headaches, and neurological pain.

Chlorophenols other than PCP

A variety of skin disorders, such as dermatitis, (chlor)acne, ulcerations and porphyria cutanea tarda are commonly observed in occupational settings. The occurrence and severity of these dermatological lesions are partly ascribed to other agents. In addition to dermal (and sometimes respiratory) symptoms and increased blood serum and urine chlorophenol levels, a variety of systemic effects has been reported, such as haematological changes, liver and kidney function changes, and neurological changes.
Very little is known on exposure levels that may result in the aforementioned effects. In unacclimated persons, an airborne T3CP concentration of 4,000 $\mu g.m^{-3}$ caused irritation effects. In two subgroups of sawmill workers ("airborne exposure" and "airborne-plus-dermal exposure", repectively) exposed to average airborne T4CP concentrations of about 3 $\mu g.m^{-3}$, blood serum T4CP levels were 4 and 8 times higher than that in controls, respectively. The only effects reported, were a productive cough and a reduced rate of forced exhalation, in the airborne-exposure group. Because these effects were not observed in the airborne-plus-dermal exposure group, these effects can not be attributed to T4CP per se (WHO, 1989).

Occupational exposure - carcinogenic and genotoxic effects

PCP

In some epidemiological studies an association has been found between exposure to mixtures of chlorophenols, not specifically PCP, and the incidence of soft tissue sarcomas, nasal and nasopharyngeal cancers, and lymphomas. However, other epidemiological studies did not indicate a significant association. Interpretation of these contradictory results is hampered by the lack of quantitative data on exposure levels and by the simultaneous exposure to other compounds, such as phenoxy acetic acids (WHO, 1987). There are also a few case reports on workers employed in a PCP production plant (for 13 or 21 years) or in a fence-installation company

which suggest an association between exposure to PCP and the occurence of Hodgkin's disease and non-Hodgkin's lymphomas (Greene et al., 1978; Bishop and Jones, 1981). However, because of the very limited number of cases, the simultaneous exposure to impurities and other chemicals, and the occurence of Hodgkin's disease in non-exposed relatives of the woodworkers, these data are inadequate for establishing a correlation.

Genotoxic effects in workers exposed to PCP were investigated in three limited studies.

In the first study, among workers in a wood treatment plant, the frequency of chromosomal aberrations (gaps and breaks) in peripheral blood cells of workers exposed to a wide range of PCP concentrations (0.005 to 15 $\mu g.m^{-3}$; mean level 1 $\mu g.m^{-3}$) appeared to be slightly increased compared to that in unexposed workers; however, the difference in the means was not statistically significant at $p < 0.05$ (Wyllie et al., 1975). It must be noted that the number of workers involved (6 exposed and 4 control workers only) and the number of 25 blood cells studied per subject are too limited to be conclusive.

In the second study, among a small group (n = 22, all smokers) of male workers in a PCP and NaPCP producing plant, the frequencies of structural chromosomal aberrations (chromatid breaks, acentric fragments, dicentrics) and sister chromatid exchanges in peripheral lymphocytes were compared with that of 22 unexposed male controls (9 smokers and 13 non-smokers). In the PCP working place, 18/67 and 10/67 measurements during the last three years showed exposure concentrations < 100 $\mu g.m^{-3}$ and > 500 $\mu g.m^{-3}$, respectively. Similarly, in the NaPCP working place, 7/55 and 8/55 measurements showed exposure concentrations of < 100 $\mu g.m^{-1}$ and > 500 $\mu g.m^{-3}$, respectively. The mean blood and urinary PCP concentrations in PCP workers were 4.7 and 2.4 $mg.l^{-1}$, respectively; in NaPCP workers the corresponding values were 2.2 and 0.8 $mg.l^{-1}$, respectively. In the group of exposed workers (n = 22) the frequency of SCEs was significantly ($p = 0.005$) increased compared to the total control group (n = 22) group of unexposed, but compared to the smoking controls (n = 13) the difference was not significant. However, the frequency of cells with structural chromosomal changes in the group of exposed workers was significantly increased compared to the smoking controls. These changes were predominantly "acentrics" (terminal deletions, acentric rings and minutes) and "dicentrics"; these changes both were increased significantly. The number of cells per subject examined was 300 and 500 in workers and controls, respectively (Bauchinger et al., 1982).

In the third study, among a small group (n = 20; 14 smokers, 6 non-smokers) of healthy workers in a production plant of PCP-containing wood preservatives, the correlation between chromosome aberrations (tetraploids, gaps, chromatid breaks, chromosome breaks, acentric fragments, dicentrics, quadriradials, translocations) and sister chromatid exchanges in peripheral blood lymphocytes on the one hand and serum PCP levels and exposure time on the other was studied. The workers had been exposed to airborne PCP concentrations ranging from 1.2 to 180 $\mu g.m^{-3}$ for 3 to 34 years. Serum PCP levels ranged from 23 to 775 $\mu g.l^{-1}$. The workers either handled dry dust of 96%-pure PCP and of 85%-pure "technical-grade" NaPCP, or handled the finished PCP solutions. The number of cells per subject examined was 60 to 100. There was no relationship between the mean frequency of SCEs and serum PCP level or time of employment. Similarly, there was no relation between the frequency of chromosomal aberrations and exposure. An effect of smoking on the frequency of SCEs, or a difference in frequency between the two subgroups was not observed either (Ziemsen et al., 1987).

Chorophenols other than PCP

Some epidemiological studies indicate an association between exposure to chlorophenols (especially T3CP) and the incidences of malignant tumours, but the results from these studies could not be confirmed in other epidemiological studies (for additional data, see PCP) (WHO, 1987, 1989).

Non-occupational exposure

PCP

In a study among 250 persons of 104 families exposed to elevated indoor PCP concentrations (exceptionally 10-25 $\mu g.m^{-3}$; frequently 2-10 $\mu g.m^{-3}$; average and median concentration 6 and 5 $\mu g.m^{-3}$, respectively) due to the use of wood preservatives, urinary PCP levels were correlated with airborne PCP levels (see 1.1.1). Measurements of a variety of biochemical parameters related to liver, kidney and blood function did not indicate a clear relationship between health status and elevated exposure. However, a number of aspecific effects such of headache, fatigue, inflammations of tonsils and mucous membranes, and hair loss may have been caused by PCP or impurities in the wood preservatives used (Krause and Englert, 1980). In another study among non-occupationally exposed persons (n = 108), a high correlation was observed between serum PCP levels and the severity of signs

and symptoms of "chronic poisoning" due to exposure to PCP and lindane containing wood preservatives. At an average serum PCP level of 13 (range 0-30) $\mu g.l^{-1}$, the majority of the subjects examined showed no or "moderate" (fatigue, headache, dizziness) symptoms of poisoning. At an average serum PCP level of 48 (range 30-100) $\mu g.l^{-1}$, about 50% and 10% of the subjects examined showed "more severe" (persistent acne, inflammation of upper respiratory tract) and "very severe" (emaciation, tachycardia, abdominal and thoracic pain, abnormal blood pressure, blood in urine) symptoms of poisoning, respectively. At an average serum PCP level of 450 (minimum level 100) $\mu g.l^{-1}$, 15% and 85% of the subjects examined showed "severe" and "very severe" symptoms of poisoning, respectively. The correlation between urinary PCP levels and the severity of the symptoms observed was less evident (Janssens and Schepens, 1985).

Brandt et al. (1977) described a case of a woman exposed to very high indoor concentrations of PCP (up to about 500 $\mu g.mg^{-3}$) for 7 years, due to the use of wood preservatives. During the first years of exposure, the woman suffered from mental and physical fatique, rhinitis, and eczematous changes on the head. Next to these effects, heachache, vomiting and loss of weight occurred at prolonged exposure. Clinical investigations during the last 5 years of exposure showed liver damage (elevated activities of the enzymes γ-GT, GOT, GPT, GLDH and LDH; cirrhosis, necrosis and inflammation) which deteriorated at prolonged exposure.

Chlorophenols other than PCP

Information on general population exposure to chlorophenols (other than PCP) specifically is not available.

Summary and conclusions "long-term exposure"

Animal data - oral exposure

One or more "life-time" carcinogenicity and toxicity studies are available for 2-MCP, 2,4-DCP, 2,4,6-T3CP and PCP. Additionally, a number of semichronic toxicity studies, exposure time 3 months to 8 months, are available for 2,4-DCP and, especially, different grades of PCP. For the remaining chlorophenols (semi)chronic studies are not available, with exception of one 3-mo study with 2,4,5-T3CP.

In most of these studies, the following toxicity parameters have been studied: survival, body and organ weights, gross pathology and

histopathology. In a number of studies additional parameters such as haematology, clinical chemistry, immunocompetence, and the activity of hepatic enzymes have been studied. Therefore, no-effect-levels for individual compounds may differ significantly. Some studies included effects on reproductive performance and non-carcinogenic effects on the progeny exposed both pre- and postnatally; these effects are already described in section 1.2.2.

Non-carcinogenic effects

Studies with 2,4-DCP, 2,4,5-T3CP and 2,4,6-T3CP primarily resulted in histo(patho)logical changes in the liver. In a study with 2,4-DCP and in the study with 2,4,5-T3CP changes were also observed in bone marrow and kidneys, respectively. Based on all non-carcinogenic effects studied, a NO(A)EL of 120 $mg.kg^{-1}$ $bw.day^{-1}$ was derived for 2,4-DCP (purity > 99%) and a NO(A)EL of 50 $mg.kg^{-1}$ $bw.day^{-1}$ for 2,4,5-T3CP (purity > 99%). The lowest dose of 2,4,6-T3CP tested, 250 $mg.kg^{-1}$ $bw.day^{-1}$, resulted in decreased body weights.

Comparative studies with different grades of PCP show that exposure to "technical-grade" PCP (high-impurity grade) results in a number of effects which are not observed at exposure to "pure" PCP at similar dose levels, or which at exposure to the latter compound are observed only at considerably higher dose levels. This is consistent with the impurities, especially polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) which are present in technical PCP-formulations. Therefore, the data on "pure" PCP and "technical-grade" PCP are discussed separately. Based on effect-levels and no-effect-levels observed in the different studies, all PCP-formulations with a total PCDF and PCDD content up to 30 ppm are considered to be "pure". Therefore, "pure" PCP includes, amongst other formulations, "Dowicide EC-7".

"Pure" PCP

Studies with "pure" PCP primarily resulted in histo(patho)logical effects in the liver and increased liver weights, at dose levels of 10 to 25 $mg.kg^{-1}$ $bw.day^{-1}$. Studies including relatively low dose levels resulted in NO(A)ELs of 3 to 5 $mg.kg^{-1}$ $bw.day^{-1}$, depending on dose levels tested.

"Technical-grade" PCP

Most marked changes observed in studies with "technical-grade" PCP include effects on hepatic enzymes (increased activity of aryl hydrocarbon hydroxylase; increased content and a shift in spectral characteristics of

cytochrome P450) and the occurence of hepatic porphyria; these effects are consistent with the high PCDF and PCDD content, compounds which similarly causes these effects. Additionally, immunosuppression appears to be associated with these impurities. The lowest dose tested, 1 $mg.kg^{-1}$ $bw.day^{-1}$ already resulted in increased activities of hepatic enzymes (aryl hydrocarbon hydroxylase and, especially, glucuronyl transferase).

Carcinogenic effects

"Life-time" carcinogenicity studies with B6C3F1 mice and/or F344 rats were conduced with 2,4-DCP (purity > 99%), 2,4,6-T3CP ("Omal", purity 96%) and PCP (both "Dowicide EC-7" and "technical-grade" PCP); the test compounds were administered in feed. The studies with 2,4-DCP were negative (no compound-related increases in malignant or benign tumours). The studies with 2,4,6-T3CP showed dose-related increases in both hepatocellular carcinomas and hepatocellular adenomas in male and female mice, and in (monocytic) leukemias in male rats (It is noted that these types of tumours were also observed in the respective control animals, and in other control animals of these strains). The carcinogenicity of the 2 PCP-formulations was studied only in mice. In both studies, dose-related increases in hepatocellular carcinomas, hepatocellular adenomas and benign adrenal medullary pheochromocytomas were observed in males. In the study with "Dowicide EC-7", dose-related increases in hepatocellular adenomas, benign adrenal pheochromocytomas, and hemangiosarcomas in spleen and liver were observed in females, while in the study with "technical-grade" PCP the incidence of tumours other than hemangiosarcomas was not increased in females. Based on these studies and similar studies with either HxCDD or 2,3,7,8-TCDD (compounds which can be present in PCP formulations) it is concluded that PCP itself is carcinogenic in B6C3F1 mice.

Limited carcinogenicity studies with other strains of mice (2-MCP, 2,4-DCP, 2,4,6-T3CP, PCP) and rats (PCP) show no indications for carcinogenicity of these compounds, with exception of the study with 2,4,6-T3CP.

Based on all data available it is concluded that there is "no evidence" and "insufficient evidence" for the carcinogenicity of 2,4-DCP and PCP, respectively, in experimental animals. Further, there is "sufficient evidence" for the carcinogenicity of 2,4,6-T3CP in experimental animals. Data on other compounds are not available or inadequate for evaluation.

Animal data - exposure by inhalation

Exposure of rats and rabbits for 4 months (4 hours per day only) to airborne PCP concentrations of 3 $mg.m^{-3}$ resulted in "minor" effects on liver function, cholinesterase activity and blood sugar. A similar exposure of rabbits to an airborne NaPCP concentration of 3 $mg.m^{-3}$ resulted in an increased liver weight; for rats this dose level appeared to be without effect.
Data on chlorophenols other than PCP are not available.

Human data

Occupational exposure

At prolonged occupational exposure to technical formulations of PCP and other chlorophenols, persistent skin lesions and respiratory disorders are most prominent effects. Additionally, effects include haematological, biochemical and immunological effects, and effects on liver and kidney function. Data on effect levels are very limited. At exposure levels up to 200 μg $PCP.m^{-3}$, serious health effects appear not to be evident. However, persistent skin lesions and respiratory disorders may occur at PCP levels below 50 $\mu g.m^{-3}$.
Some epidemiological studies and case reports suggest an association between exposure to chlorophenols and the incidence of soft tissue sarcomas, nasal and nasopharyngyal cancers, and lymphomas. However, this association is not confirmed in other studies.
In 1 out of 3 very limited studies for genotoxic effects in workers exposed to PCP, a significantly increased incidence of chromosomal aberrations ("acentrics" and "dicentrics") was observed. However, these studies are inadequate for evaluation.

Non-occupational exposure

Limited data on indoor exposure to PCP indicate that exposure to 2 to 10 μg $PCP.m^{-3}$ does not result in measurable effects on health status. However, non-specific effects may occur at these dose levels.

1.2.4 Genotoxicity

In vitro studies

There are only 6 chlorophenols that have been studied for gene mutation in at least one prokaryotic test system (bacteria) and one eukaryotic test system (mammalian cells and/or yeast). The tests for gene mutation and additional *in vitro* genotoxicity tests with these compounds (2,4-DCP, 2,6-DCP, 2,4,5-T3CP, 2,4,6-T3CP, 2,3,4,6-T4CP and PCP) are summarized in table 1.5. In the text below, the main data on these compounds are discussed, and, additionally, data on the remaining 13 chlorophenols which have been studied for gene mutation only in the *Salmonella/* mammalian-microsome assay.

Gene mutations in vitro

Within the United States National Toxicology Program, all 19 chlorophenols have been studied in a modified Ames test *(Salmonella/* mammalian-microsome assay), resulting in negative responses for all compounds (NTP, 1989a,b). The original data of these tests have been published by Haworth et al. (1983) and Zeiger et al. (1988), with exception of the tests with 4 trichlorophenols (2,3,4-T3CP, 2,3,5-T3CP, 2,3,6-T3CP and 3,4,5-T3CP). Each compound was tested with 4 *Salmonella typhimurium* strains (namely TA98, TA100, TA1535 and TA1537 [Haworth et al., 1983] or strains TA97, TA98, TA100 and TA1535 [Zeiger et al., 1988]), both in the absence and presence of a metabolic activation system. A microsomal S9-fraction from rat or hamster liver was used as activation system. Only in one test with 2,4-DCP, namely, in the test with strain TA1535 in the presence of hamster S9-mix, an equivocal response was observed. All other tests were negative, regardless of compound tested, test strain and metabolic activation.
The negative response of 2,4-DCP, 2,6-DCP, 2,4,5-T3CP, 2,4,6-T3P and PCP in the *Salmonella/* mammalian-microsome assay has been confirmed in 4, 2, 2, 2 and 5 independent studies, respectively. In most of these studies, tests were conducted with at least 4 *S. typhimurium* strains, both in the absence and presence of metabolic activation (Anderson et al., 1972; Buselmaier et al., 1972; Shirasu, 1975; Rasanen et al., 1977; Simmon et al., 1977; Nestman et al., 1980; Rapson et al., 1980; Probst et al., 1981; Moriya et al., 1983, see also table 1.5).
On the other hand, a positive response for both 2,4,5-T3CP and 2,4,6-T3CP was observed in one study using the *Salmonella/* mammalian-microsome assay;

in this study a positive response was observed in 3 out of 4 test strains used (TA97, TA98, TA100, TA104). All but one positive responses were observed in the presence of metabolic activation (Strobel and Grummt, 1987). It is noted that the purities of the test compounds were not reported in the study by Strobel and Grummt (1987). Another study with this test system resulted in an equivocal response for PCP, in 1 out of 2 test strains used (TA98, TA100). The equivocal response was observed in the presence of metabolic activation (Nishimura et al., 1982).
The negative response of the remaining 13 chlorophenols (which are not listed in table 1.5) in the *Salmonella* /mammalian-microsome assay has been confirmed in one or two studies. In the one study, all 6 DCP, 4 out of 6 T3CP (2,3,5-T3CP, 2,3,6-T3CP, 2,4,5-T3CP and 2,4,6-T3CP) and 2,3,4,6-T4CP were tested with 4 different test strains: TA98, TA100, TA1535 and TA1537, both in the absence and presence of metabolic activation (Rasanen et al., 1977). In the other study, all 3 MCP, all 6 DCP, and 3 out of 6 T3CP (2,3,4-T3CP, 2,3,5-T3CP and 2,4,6-T3CP) were tested in strain TA100; the presence of metabolic activation has not been stated (Rapson et al., 1980). It is noted that no duplicate tests were performed in the latter study.
On the other hand, one study resulted in positive responses for 3-MCP, 4-MCP and 2,3,6-T3CP in 2 or 3 out of 4 test strains used (TA97, TA98, TA100, TA104). In most cases a positive response was observed only in the presence of metabolic activation (Strobel and Grummt, 1987).

Five out of the 6 chlorophenols listed in table 5.1 have also been studied for gene mutations in one or more tests with bacterial species other than *S. typhimurium* or in yeast. In one of these studies, exposure of yeast *Saccharomyces cerevisiae* to 2,4,6-T3CP resulted in an equivocal response for gene mutation, while exposure to PCP resulted in a positive reponse for both gene mutation and mitotic gene conversion (Fahrig et al., 1978). Additionally, PCP showed a positive response for mitotic gene conversion in a second study with *S. cerevisiae* (Fahrig, 1974); the positive response was observed at a concentration that was lethal to 70% of the cells exposed. The remaining tests for gene mutations in bacteria or yeast resulted in negative responses (Buselmaier et al., 1972; Fahrig, 1974; Shirasu, 1975; Probst et al., 1981; Moriya et al., 1983; Nestmann and Lee, 1983).

The 6 chlorophenols listed in table 1.5 have also been studied for gene mutation in one or two types of mammalian cells.

In a study with V79 Chinese hamster cells, tests with 2,4-DCP, 2,6-DCP, 2,4,5-DCP, 2,4,6-T3CP, 2,3,4,6-T4CP and PCP resulted in a negative response (Jansson and Jansson, 1986). In a second study with V79 Chinese hamster cells, using a similar and modified test protocol, a negative response was observed for 2,6-DCP (2 tests) and PCP (1 test). The result for the other two chlorophenols tested, 2,4,6-T3CP and 2,3,4,6-T4CP was equivocal: for both compounds 1 test resulted in a positive response, while a negative response was observed in 1 or 2 tests using a different test protocol (Hattula and Knuutinen, 1985).
Mouse L5178Y lymphoma cell assays with 2,4-DCP and 2,4,6-T3CP resulted in positive, reproducible responses for forward mutation, in the absence of metabolic activation. This test was not conducted in the presence of metabolic activation (McGregor et al., 1988; NTP, 1989a).

Chromosomal aberrations in vitro

Within the United States National Toxicology Program, 7 chlorophenols were studied in a test for chromosomal aberrations, using cultured hamster ovary (CHO) cells. Each compound was tested both in the absence and presence of a metabolic activation system (microsomal S9-fraction from rat liver). A positive response was reported for 2,3,4-T3CP, 2,3,6-T3CP, 2,3,5,6-T4CP and PCP, but not for 2,4-DCP, 2,4,6-T3CP and 3,4,5-T3CP (NTP, 1989a,b). However, based on the original data, the "positive" response reported for PCP in the presence of metabolic activation is considered to be "equivocal" (see table 1.5). The original data with regard to 2,3,4-T3CP, 2,3,6-T3CP, 3,4,5-T3CP and 2,3,5,6-T4CP are not available; therefore, the results reported for these compounds cannot be evaluated.
According to Fahrig (1974), PCP showed an equivocal response for chromosomal aberrations in a test with human lymphocytes. The original data on this test are not reported; therefore, this result cannot be evaluated.
Sodium pentachlorophenate, NaPCP, has also been studied in a test for chromosomal aberrations in human lymphocytes; this test resulted in a negative response (Ziemsen et al., 1987).

Other genotoxic effects in vitro

The aforementioned 7 chlorophenols (see "chromosomal aberrations *in vitro*) were also studied in a test for SCEs (sister chromatid exchanges), using CHO cells. A positive response was reported for 2,4-DCP, 2,3,4-T3CP, 2,3,5,6-T4CP and PCP, but not for 2,3,6-T3CP, 2,4,6-T3CP and 3,4,5-T3CP

(NTP, 1989a,b). However, based on the original data, the "positive" responses reported for 2,4-DCP and PCP are considered to be "equivocal" and "negative", respectively (see Table 1.5). The original data with regard to 2,3,4-T3CP, 2,3,6-T3CP, 3,4,5-T3CP and 2,3,5,6-T4CP are not available; therefore, the results reported for these compounds cannot be evaluated.
Sodium pentachlorophenate, NaPCP, has been studied in a test for SCEs in human lymphocytes; this test resulted in a negative response (Ziemsen et al., 1987).
In rat hepatocytes exposed to 2,4-DCP, unscheduled DNA-synthesis was not affected (Probst et al., 1981).

In vivo studies

In vivo genotoxicity tests with animal species are summarized in table 1.6. Tests for SCEs in bone marrow cells of mice exposed for 1 day to a single toxic (sublethal) dose of 2,3-DCP, 2,4-DCP, 2,5-DCP, 2,6-DCP or PCP resulted in negative responses (Kessler et al., abstract). Tests in which mice were exposed for 2 weeks to 2-MCP or 2,4-DCP at dose levels up to 175 and 638 $mg.kg^{-1}$ $bw.day^{-1}$, respectively, did not result in effects on sperm morphology, testicular DNA synthesis, SCEs in testis and bone marrow, and mitotic index in bone marrow (Borzelleca, 1985c; see also table 1.2).
A spot test in which mice were exposed to a single intraperitoneal dose of 100 mg 2,4,6-T3CP.kg^{-1} bw or 100 mg PCP.kg^{-1} bw on day 10 of gestation did not result in increased incidences of spots of genetic relevance (Fahrig et al., 1978). Exposure of mice to PCP in two other tests, namely a 3-hr host-mediated assay (single intracutaneous dose of 75 $mg.kg^{-1}$ bw) and a 5-w sperm morphology assay (repeated intraperitoneal doses of 6-400 $mg.kg^{-1}$ $bw.day^{-1}$) did not result in bacterial gene mutations or effects on sperm morphology, respectively (Buselmaier et al., 1972; Osterloh et al., 1983).
A sex-linked lethal test with *Drosophila melanogaster* and another test with *Drosophila sp.* (endpoints: nondisjunction and loss of sex chromosomes) both were negative (Vogel and Chandler, 1974; Ramel and Magnusson, 1979).

Additional data

The DNA-damaging potential and cytotoxicity of PCP and its major metabolite tetrachloro-p-hydroquinone (TCH) was studied in *in vitro* tests. In tests with calf-thymus DNA, covalent binding of TCH, but not of PCP, was found. In tests with DNA of bacteriophage PM2, the number of single-strand breaks increased proportionally to the concentration of TCH, while PCP did not

induce breaks. Addition of superoxide dismutase and catalase to the reaction mixture strongly reduced the number of DNA strand breaks, indicating that a large proportion of the strand breaks is caused by O_2^- and/or H_2O_2, by-products of the formation of semiquinone radicals from hydroquinones such as TCH. The cytotoxicity of PCP, with and without metabolic activation by S9-mix, and of TCH was studied by determining the colony-forming ability of human fibroblasts. In the presence of metabolic activation, the cytotoxicty of PCP increased; in this case TCH was identified in the incubation mixture, suggesting that the formation of this metabolite is responsible for the increased cytotoxicity. This was confirmed in tests in which TCH was added directly to the medium and was found to be more cytotoxic than PCP, at equimolar concentrations. The results of this study indicate that the metabolite TCH is able to bind to DNA and to cause DNA strand breaks, while PCP itself does not. This suggests that TCH plays a role in the cytotoxicity of PCP (Witte et al., 1985).

In a modified *Allium* test, bulbs of onion *A. cepa* were exposed in hydroculture for 5 days to concentrations of 0.5 to 50 $mg.l^{-1}$ 2,4-DCP or 2,4,5-T3CP. In addition to a dose-related decrease in mitotic index, chromosome damage (e.g. increased incidences of abnormal metaphases, delayed anaphases, sticky stages of mitosis, and bridges and fragments) were observed. Chromosome damage was most obvious at toxic (parameter: root growth inhibition) concentrations ≥ 5 $mg.l^{-1}$ (Fiskesjö et al., 1981).

Summary and conclusions "genotoxicity"

In vitro studies

There are only 6 chlorophenols (2,4-DCP, 2,6-DCP, 2,4,5-T3CP, 2,4,6-T3CP, 2,3,4,6-T4CP, and PCP) that have been studied for gene mutation in at least one prokaryotic test system (bacteria) and one eukaryotic test system (mammalian cells and/or yeast). Three of these 6 compounds, namely 2,4-DCP, 2,4,6-T3CP and PCP, were also studied in tests for allied genotoxic effects, primarily chromosomal aberrations and sister chromatid exchanges (SCEs) in mammalian cells. For 5 out of these 6 compounds (2,6-DCP excepted), one or more equivocal and/or positive results were observed. However, the majority of the tests with these compounds resulted in negative responses.

The majority of the remaining 13 chlorophenols was studied only for gene mutation in one test system, the *Salmonella/* mammalian-microsome assay. One study resulted in a positive response for 3-MCP, 4-MCP and 2,3,6-T3CP, but this result is contradicted by the results of at least one other study using this test system.

In vivo studies

Mammalian tests with 2-MCP, 2,3-DCP, 2,4-DCP, 2,5-DCP, 2,6-DCP and PCP for effects such as bone marrow SCEs, sperm morphology and/or testicular DNA synthesis resulted consistently in negative responses, with exception of one test with 2,5-DCP which resulted in an equivocal response with regard to bone marrow SCEs after a single toxic dose. A mammalian spot test with 2,4,6-T3CP and PCP also resulted in negative responses, as well as a host-mediated assay in mice.

A sex-linked lethal test with *Drosophila melanogaster* and a test for nondisjunction and loss of sex chromosomes in this insect species both were negative.

In a modified *Allium* test (plant), chromosome damage was induced by 2,4-DCP and 2,4,5-T3CP. However, the effects were most obvious at toxic concentrations that resulted in root growth inhibition.

Conclusion genotoxicity

On the basis of all data available it is concluded that there is insufficient evidence for mutagenicity of 2,4-DCP, 2,6-DCP, 2,4,5-T3CP, 2,4,6-T3CP, 2,3,4,6-T4CP, and PCP. With regard to the chlorophenols remaining, the available data are inadequate for evaluation.

Table 1.1 Acute toxicity studies with chlorophenols

Route of administration	Animal species	* LD50 mg/kg bw		Reference(s)
2-MCP				
oral	mouse	345 / 670	(n = 2)	Borzelleca et al., 1985a; WHO, 1989
oral	rat	670		RTECS, 1989
oral	"mammal"	440		RTECS, 1989
i.p.	mouse	235		RTECS, 1989
i.p.	rat	230		RTECS, 1989
s.c.	rat	950		RTECS, 1989
3-MCP				
oral	mouse	520		Borzelleca et al., 1985a
oral	rat	570 / 670	(n = 2)	Borzelleca et al., 1985a; RTECS, 1989
i.p.	rat	355		RTECS, 1989
s.c.	rat	1,390		RTECS, 1989
4-MCP				
oral	mouse	365 / 1,400	(n = 2)	Borzelleca et al., 1985a; RTECS, 1989
oral	rat	260		RTECS, 1989
oral	"mammal"	500		RTECS, 1989
i.p.	mouse	330		RTECS, 1989
i.p.	rat	280		RTECS, 1989
s.c.	rat	1,030		RTECS, 1989
dermal	rat	1,500		RTECS, 1989
dermal	"mammal"	1,000		RTECS, 1989
inhalation	rat	11	LC50, mg/m^3	RTECS, 1989
2,3-DCP				
oral	mouse	2,375		Borzelleca et al., 1985a
2,4-DCP				
oral	mouse	1,280 - 1,630	(n = 4)	Borzelleca et al., 1985a,b; NTP, 1989a
oral	rat	580 - 4,000	(n = 4)	Borzelleca et al., 1985b; NTP, 1989a
oral	"mammal"	465		RTECS, 1989
i.p.	mouse	155		
i.p.	rat	430		Borzelleca et al., 1985b; NTP, 1989a
s.c.	rat	1,730		Borzelleca et al., 1985b; NTP, 1989a
dermal	"mammal"	790		RTECS, 1989
2,5-DCP				
oral	mouse	945		Borzelleca et al., 1985a
oral	rat	580		RTECS, 1989
2,6-DCP				
oral	rat	2,940		RTECS, 1989
oral	mouse	2,120		Borzelleca et al., 1985a
i.p.	rat	390		RTECS, 1989
s.c.	rat	1,730		RTECS, 1989
3,4-DCP				
oral	mouse	1,685		Borzelleca et al., 1985a

(to be continued)

Table 1.1 Acute toxicity studies with chlorophenols (continued)

Route of administration	Animal species		LD50 mg/kg bw			Reference(s)
3,5-DCP						
oral	mouse		2,390			Borzelleca et al., 1985a
2,3,6-T3CP						
oral	rat		820			Strobel and Grummt, 1987
oral	guinea pig		1,000			Strobel and Grummt, 1987
i.p.	rat		310 / 355	(n = 2)		Strobel and Grummt, 1987; RTECS, 1989
i.v.	mouse		56			Strobel and Grummt, 1987
s.c.	rat		2,260			Strobel and Grummt, 1987
2,4,5-T3CP						
oral	mouse		600			RTECS, 1989
oral	rat		620 - 2,960	(n = 3)		RTECS, 1989; McCollister et al., '61
oral	guinea pig		1,000			RTECS, 1989
i.p.	rat		355			RTECS, 1989
i.v.	mouse		56			RTECS, 1989
s.c.	rat		2,260			RTECS, 1989
2,4,6-T3CP						
oral	rat		820			RTECS, 1989
oral	"mammal"		455			RTECS, 1989
i.p.	rat		275			RTECS, 1989
dermal	"mammal"		700			RTECS, 1989
3,4,5-T3CP						
i.p.	rat		370			RTECS, 1989
2,3,4,5-T3CP						
oral	mouse		140 - 530	(n = 5)		Ahlborg & Larsson, 1978; Borzelleca et al., 1985c
i.p.	mouse		95 / 130	(n = 2)		Ahlborg & Larsson, 1978
dermal	rat	[3]	2,000		MLD	Shen et al., 1983
2,3,4,6-T4CP						
oral	mouse		130 - 735	(n = 4)		Ahlborg & Larsson, 1978
oral	rat		140 / 360	(n = 2)		RTECS, 1989; WHO, 1989
oral	guinea pig		250			RTECS, 1989
i.p.	mouse		80 - 250	(n = 3)		Ahlborg & Larsson, 1978; WHO, 1989
i.p.	rat		130			RTECS, 1989
s.c.	mouse		120			WHO, 1989
s.c.	rat		210			WHO, 1989
dermal	rat		485			RTECS, 1989
dermal	rabbit		250			RTECS, 1989
2,3,5,6-T4CP						
oral	mouse		90 - 980	(n = 4)		Ahlborg & Larsson, 1978
i.p.	mouse		50 / 110	(n = 2)		Ahlborg & Larsson, 1978
dermal	rat	[4a]	2,000		MLD	Shen et al., 1983
dermal	rat	[4b]	500			Shen et al., 1983
dermal	rat	[4c]	300			Shen et al., 1983

(to be continued)

Table 1.1 Acute toxicity studies with chlorophenols (continued)

Route of administration	Animal species	* LD50 mg/kg bw		Reference(s)
PCP				
oral	mouse	35 - 295	(n = 8)	Ahlborg & Larsson, 1978; Borzelleca et al., 1985a; WHO, 1987
oral	rat	25 - 175	(n = 5)	Borzelleca et al., 1985a; WHO, 1987a
oral	rat [1]	65 - 205	(n = 3)	Schwetz et al., 1974b, 1978
oral	hamster	170		RTECS, 1989
oral	guinea pig	100		Knudsen et al., 1974
oral	rabbit	70 - 130	(n ≥ 4), MLD	WHO, 1987
oral	duck	380		RTECS, 1989
oral	dog	100		Knudsen et al., 1974
oral	sheep	120	MLD	WHO, 1987
oral	calf	140	MLD	WHO, 1987
i.p.	mouse	30 - 60	(n = 4)	WHO, 1987
i.p.	rat	55		RTECS, 1989
s.c.	mouse	80		WHO, 1987
s.c.	rat	40 - 100	(n = 3)	WHO, 1987
s.c.	hamster	70 - 85	(n ≥ 2)	WHO, 1987
s.c.	rabbit	70 - 85	(n ≥ 2)	WHO, 1987
dermal	rat	95 - 330	(n = 4)	WHO, 1987; RTECS, 1989
dermal	rabbit	40 - 350	(n ≥ 6), MLD	WHO, 1987
inhalation	mouse	225	**LC50**, mg/m^3	RTECS, 1989
inhalation	rat	355	**LC50**, mg/m^3	RTECS, 1989
NaPCP				
oral	rat	70 - 210	(n ≥ 4)	WHO, 1987
oral	rabbit	220 - 700	(n ≥ 5), MLD	WHO, 1987
i.p.	rat	35		Hoben et al., 1976a
i.p.	rabbit	50 - 150	(n ≥ 2), MLD	WHO, 1987
i.v.	rabbit	22 - 23	(n ≥ 3), MLD	WHO, 1987
s.c.	mouse	85		WHO, 1987
s.c.	rat	40 - 65	(n = 2)	WHO, 1987
s.c.	rabbit	100 - 300	(n ≥ 3), MLD	WHO, 1987
s.c.	dog	135	MLD	WHO, 1987
dermal	rat	105		WHO, 1987
dermal	Guinea pig	265	MLD	WHO, 1987
dermal	rabbit	250 - 600	(n ≥ 4), MLD	WHO, 1987
inhalation (2 h)	rat	295	**LC50**, mg.m^3	WHO, 1987
inhalation	rat [2]	12		Hoben et al., 1976b
TCH				
oral	mouse	500		Ahlborg & Larsson, 1978
i.p.	mouse	35		Ahlborg & Larsson, 1978

i.v. = intravenous; i.p. = intraperitoneal; s.c. = subcutaneous

n = number of values available

* LD50, unless stated otherwise; LD50 in mg/kg bw
MLD: Minimum Lethal Dose in mg/kg bw (LD50 not available)

For further footnotes, see next page.

[1] Unpublished data Dow Chemical Company. Test compound: Dowicide EC-7 (90% PCP; 10% T4CP; relative low content of nonphenolic impurities). LD50-values were 65, 135, and 205 mg/kg bw for 3-4 d old animals, adult females, and adult males, respectively.

[2] Exposure to an aqueous aerosol. The LD50 (mg/kg bw) has been calculated by the investigators; the LC50 is not reported.

[3] The undissociated compound and the sodium phenate were studied in separate tests.

[4] a) undissociated compound, purified; b) undissociated compound, commercial product; c) sodium phenate.

[5] Metabolite of 2,3,5,6-T4CP and PCP.

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2-MCP (purity not reported)					
mouse CD-1 ICR m,f adult	0 (c)-0 (v-c)-35-69-175 mg/kg bw/day by gavage. Vehicle: corn oil (12 animals of each sex/group)	2-w	69	35	Borzelleca, 1985c
Parameters: Mortality, body weight, organ weights and ratios, gross pathology at necropsy, haematology, clinical chemistry, immune response, hepatic microsomal MFO activity, genotoxicity (testicular DNA synthesis, sperm morphology, sister chromatid exchanges in testis and bone marrow, mitotic index in bone marrow, reproductive toxicity (in vitro penetration, fertilization, blastula formation) Results: All animals exposed to 175 mg/kg bw/day died. Body weights were reduced at 69 mg/kg bw/day.					
2,4-DCP (purity > 99%)					
mouse B6C3F1 m,f age 7 w	0-2,500-5,000-10,000-20,000-40,000 mg/kg feed (5 animals of each sex/group)	2-w	2,800*	1,400*	NTP, 1989a
Parameters: Mortality, feed consumption, body weight (growth), gross pathology at necropsy. Results: At 40,000 mg/kg feed, one of five males died; in both sexes, final body weights were lower than the initial body weights. Body weight gain in the other dose groups was similar to that in controls, although feed consumption was reduced (50%) at ≥ 20,000 mg/kg feed.					
2,4-DCP (purity not reported)					
mouse CD-1 ICR m,f adult	0 (c)-0 (v-c)-64-128-638 mg/kg bw/day by gavage. Vehicle: corn oil (12 animals of each sex/group)	2-w	.	≥ 638	Borzelleca, 1985c
Parameters: Mortality, body weight, organ weights and ratios, gross pathology at necropsy, haematology, clinical chemistry, immune response, hepatic microsomal MFO activity, genotoxicity (testicular DNA synthesis, sperm morphology, sister chromatid exchanges in testis and bone marrow, mitotic index in bone marrow, reproductive toxicity (in vitro penetration, fertilization, blastula formation) Results: The highest concentration tested did not result in effects on the majority of the parameters studied. Therefore, this dose level is considered to be the NO(A)EL, although the number of placelets and the hepatic microsomal MFO activity (glutathione, microsomal protein, cytochrome b5) were increased.					

(to be continued)

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4-DCP (purity > 99%)					
rat F344/N m,f age 6 w	0-2,500-5,000-10,000-20,000-40,000 mg/kg feed (5 animals of each sex/group)	2-w	2,000*	1,000*	NTP, 1989a
Parameters: Mortality, feed consumption, body weight (growth), gross pathology at necropsy. Results: At 40,000 mg/kg feed, final body weights relative to controls were lower than the initial body weights, while feed consumption was reduced ≥ 50%. At 20,000 mg/kg feed, final body weights relative to controls were reduced 10% (females) and 20% (males), while feed consumption by females and males was reduced 25-55% and 15%-35%, respectively.					
2,4,5-T3CP (purity 97-98%)					
rat m 270 g	18 doses by stomach tube in 24 days; dose levels 30-100-300-1,000 mg/kg bw (5 animals/dose)	3-w	750	225	McCollister et al., 1961
Parameters: Mortality, growth, final body and organ weight ratios, histology of major organs, and haematology. Results: At 1,000 mg/kg bw, a small (4%), temporary loss of body weight was observed, and a 15% increase in the weight of kidneys.					
2,4,5-T3CP (purity 97-98%)					
rabbit	20 oral doses by intubation in 28 day; dose levels 1-10-100-500 mg/kg bw (3-2-1-1 animals/dose, respectively)	4-w	?	?	McCollister et al., 1961
Parameters: "Pathologic examination" (no further data). Results: "Very slight kidney changes" and "very slight kidney and liver changes" were reported at dose levels of 100 and 500 mg/kg bw, respectively. No further data reported. These data cannot be evaluated; therefore, no LED and NO(A)EL was established.					
2,4,6-T3CP ("Dowal", "Dowicide 2S", purity 96%-97%; 17 minor contaminants [not specified]; PCDD not determined)					
mouse B6C3F1 age 6 w	0-6,800-10,000-14,700-21,500-31,500 mg/kg feed (5 animals of each sex/group)	7-w	2,100*	1,400*	NCI, 1979
Parameters: Mortality, body weight (growth), histopathology (no further data). Results: Two of 5 males and 2/5 females died at 31,500 mg/kg feed. Growth of males and females was reduced was reduced (> 10%) at 14,700 and 31,500 mg/kg feed, respectively. Histopathological changes were observed at 31,500 mg/kg feed; all tissues of animals at ≤ 21,500 mg/kg were essentially normal (no details reported).					

(to be continued)

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4,6-T3CP ("Omal", "Dowicide 2S", purity 96%-97%; 17 minor contaminants [not specified]; PCDD not determined)					
rat F344 age 6 w	0-10,000-14,700-21,500-31,500-46,000 mg/kg feed (5 animals of each sex/group)	7-w	1,470*	1,000*	NCI, 1979
Parameters: Mortality, body weight (growth), histopathology (no further data). Results: Two of 5 males and 3/5 females died at 46,000 mg/kg feed; in addition, histopathological changes (increase in splenic hematopoiesis in both sexes; midzonal vacuolation of hepatocytes in 2/5 males). Growth was reduced (> 10%) at 14,700 mg/kg feed, in both sexes.					
2,3,4,6-T4CP ("commercial-grade", purity 73%; 27% PCP)					
rat Sprague-Dawley f (nonpregnant)	0-3-10-30-100-300 mg/kg/bw/day (5 animals/group)	10-d plus recovery period	100	30	Schwetz et al., 1974a
Parameters: Mortality and body weight (recorded at 3-day intervals during the treatment period and at weekly intervals following treatment). The duration of the recovery period is not reported. Results: Mortality at 100 and 300 mg/kg/day.					
2,3,4,6-T4CP (purity > 99%)					
rat Wistar age 2 mo	0-10-50-100 mg/kg bw/day, administered intragastrically (10 animals/group ?)	8-w	50	10	Hattula et al., 1981b
Remarks: The number and sex of animals is not reported; based on a preceeding study, the number of animals/group probable is 10. Histopathological data were reported briefly. Parameters: Feed and water consumption, growth, and histopathology of the major organs. Results: Severe histopathological changes (e.g. necroses which covered most of the parenchyma) were found in the liver of at least one animal at 50 and 100 mg/kg bw/day. In the small intestine, necroses were observed in 3 animals at 100 mg/kg bw/day.					
PCP ("Dowicide EC-7"; purity and impurities not reported)					
mouse B6C3F1 f age 6-7 w	0 or 100 mg/kg bw/day, by gastric intubation (8 animals/group)	2-w	.	100	Holzapple et al., 1987
Remarks: On day 10 or day 11, groups of animals were given i.p. injections of sheep red blood cells (SRBC). Parameters: In vivo IgM antibody response of spleen cells (number of anti-SRBC antibody-forming cells producing IgM) on day 4 (peak day) and 5 after immunization. Result: No effect.					

(to be continued)

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical-grade"; purity and impurities not reported)					
mouse B6C3F1 f age 6-7 w	0-10-30-100 mg/kg bw/day, by gastric intubation (8 animals/group)	2-w	10	.	Holzapple et al., 1987

Remarks: On day 10 or day 11, groups of animals were given i.p. injections of sheep red blood cells (SRBC).
Parameters: In vivo IgM antibody response of spleen cells (number of anti-SRBC antibody-forming cells producing IgM) on day 4 (peak day) and 5 after immunization.
Result: Both the day 4 and day 5 response were dose-related decreased; the decreases were statistically significant ($p < 0.05$) at all dose levels.

PCP ("pure", purity 98.6%; 1.4% T4CP; 2,200 ppm heptachlorohydroxydibenzofuran, 1,100 ppm hexachlorohydroxydibenzofuran, 2,100 ppm nonachlorohydroxydiphenyl ether, 900 ppm octachlorohydroxydiphenyl ether, 100 ppm heptachlorohydroxydiphenyl ether, < 1 ppm OCDD, < 1 ppm HxCDD, < 1 ppm TCDD)					
mouse B6C3F1 m,f age 8-9 w	0-20-100-500-2,500-12,500 mg/kg feed; (19 males and 5 females/dose; controls: 19 males and 11 females)	4-w	70*	14*	NTP, 1989b

Parameters: Mortality, feed consumption, body weight (growth), weight of the major organs, gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined), haematology, clinical chemistry, urinalysis, and supplemental studies (aryl hydrocarbon hydroxylase, liver porphyrins, oxidative phosphorylation, cytochrome P450, body temperature).
Results: All animals exposed to 12,500 mg/kg feed and 2/19 males exposed to 2,500 mg/kg feed died. At 500 mg/kg feed there was no effect on growth; at this dose level all animals examined histologically (5/5 males and 5/5 females) showed compound-related liver lesions (necrosis, cytomegaly, karyomegaly, nuclear atypia, and degeneration). Feed consumption was similar in all groups. Most data on the other parameters are reported to a limited extend. Therefore, and because of the short exposure time, the LED and NO(A)EL indicated in this table are based on the parameters discussed in this "result" section.

(to be continued)

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("Dowicide EC-7", purity 91%; 9% T4CP; 0.2 ppm HpCDF, 0.1 ppm HxCDF, 0.7 ppm OCDD, 0.5 ppm HpCDD, 0.2 ppm HxCDD, < 0.04 ppm TCDD)					
mouse B6C3F1 m,f age 8-9 w	0-20-100-500-2,500-12,500 mg/kg feed (19 males and 5 females/dose; controls 19 males and 11 females)	4-w	70*	14*	NTP, 1989b
Parameters:	Mortality, feed consumption, body weight (growth), weight of the major organs, gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined), haematology, clinical chemistry, urinalysis, and supplemental studies (aryl hydrocarbon hydroxylase, liver porphyrins, oxidative phosphorylation, cytochrome P450, body temperature).				
Results.	All animals exposed to 12,500 mg/kg feed and more than 50% of the animals exposed to 2,500 mg/kg feed died. At 500 mg/kg feed there was no adverse effect on growth; at this concentration 2 of 5 males examined histologically showed compound-related liver lesions (necrosis, cytomegaly, karyomegaly, nuclear atypia, and degeneration). In females these liver lesions were only found at (≥) 2,500 mg/kg feed. Feed consumption of males exposed to 2,500 mg/kg feed was 80% higher than that of control males; feed consumption in all other groups was similar. Most data on the other parameters are reported to a limited extend. Therefore, and because of the short exposure time, the LED and NO(A)EL indicated in this table are based on the parameters discussed in this "result" section.				
PCP ("technical grade", purity 90%; 3.8% T4CP; 3.6% nona-, 1.9% octa- and 0.1% heptachlorohydroxydiphenyl ether; 0.5% hepta- and 0.2% hexachlorohydroxydibenzofuran; 45 ppm OCDF, 90 ppm HpCDF, 10 ppm HxCDF, 1.4 ppm PeCDF, 1,390 ppm OCDD, 300 ppm HpCDD, 10 ppm HxCDD, TCDD not quantitated)					
mouse B6C3F1 m,f age 8-9 w	0-20-100-500-2,500-12,500 mg/kg feed (19 males and 15 females/dose; controls: 19 males and 11 females)	4-w	70*	14*	NTP, 1989b
Parameters:	Mortality, feed consumption, body weight (growth), weight of the major organs, gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined), haematology, clinical chemistry, urinalysis, and supplemental studies (aryl hydrocarbon hydroxylase, liver porphyrins, oxidative phosphorylation, cytochrome P450, body temperature).				
Results:	Fourteen of 19 males and 7/15 females exposed to 12,500 mg/kg feed died. At 2,500 mg/kg feed, body weight gain of males was 40% lower than that of control males, but final body weight relative to controls was not affected. At 500 mg/kg feed, all animals examined histologically (5/5 males and 5/5 females) showed compound-related liver lesions (necrosis, cytomegaly, karyomegaly, nuclear atypia, and degeneration). Feed consumption was similar in all groups. Most data on the other parameters are reported to a limited extend. Therefore, and because of the short exposure time, the LED and NO(A)EL indicated in this table are based on the parameters discussed in this "result" section.				

(to be continued)

Table 1.2 Subacute toxicity studies with chlorophenols - oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("commercial-grade", purity 88%; 4% T4CP; 6% higher chlorinated phenoxyphenols)					
rat Sprague-Dawley f (nonpregnant)	0-3-10-30-50-70 mg/kg/bw/day (5 animals/group)	10-d plus recovery period	70	50	Schwetz et al., 1974b

Remarks: Dosing regimen not reported
Parameters: Mortality and body weight (recorded at 3-day intervals during the treatment period and at weekly intervals following treatment). The duration of the recovery period is not reported.
Results: Animals receiving 70 mg/kg bw/day lost weight during the treatment period.

PCP ("pure", purity > 99%; 170 ppm OCDD; 4 ppm HpCDD; < 1 ppm HpCDF; < 1 ppm OCDF; other PCDD and PCDF at the ppb level)					
rat Wistar f 150 g	0 or 500 mg/kg feed; dose level equal to 40 mg/kg bw/day (20 animals/group)	8-w		40	Debets et al., 1980

Remarks: Interim sacrifices of 4 animals/group after 1, 2, 4 and 6 weeks of exposure.
Parameters: Mortality, growth, liver weight, microsomal liver enzymes (cytochrome P-450, p-nitroanisole O-demethylase, aminopyrine N-demethylase, NADPH-cytochrome c reductase, p-nitrophenol glucuronyl transferase and ethoxyresorufin O-de-ethylase), total urinary porphyrin and urinary porphyrin pattern.
Result: The treatment caused an increase in activity of (specific cytochrome P-448-mediated) ethoxyresorufin O-demethylase (20-fold) and glucuronyl transferase (3-fold), and a blue shift in the Soret maximum of the reduced hepatic cytochrome P-450--CO complex, of 0.5 nm.
The NO(A)EL indicated is based on the parameters mortality, growth and liver weight, because these endpoints are considered to be more relevant in subacute studies than the other parameters studied.

LED: Lowest-effect-dose
NO(A)EL: No-observed-(adverse)-effect-level

* Feed studies: standard "Conversion Factors" (mg/kg in feed : CF = mg/kg bw/day) of 7 and 10 have been used for mouse and rat, respectively.

Table 1.3 Reproductive toxicity studies with chlorophenols - (long-term) oral exposure

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2-MCP (purity 97%; impurities not reported)					
rat Sprague Dawley f age 3 w	0-5-50-500 mg/l drinking water, equivalent to 0-0.5-5-50 mg/kg bw/day (12-14 animals/group)	± 6-mo (pre- and postnatal exposure of progeny)	50**	5**	Exon & Koller, 1982,1983a,b, 1985
Remarks:	Dams were exposed from 3 w of age through gestation (bred at 90 d) and lactation. Eight randomly selected pups from each group were weaned at 3 w of age and continued on treatment for 10-15 weeks. The design and results of this study have been reported in different publications which contain some inconsistencies. The results presented in this table have been derived from all data available.				
Parameters:	Maternal toxicity (body weight gain prior to breeding), reproductive performance (conception, litter size [live and stillborn], number of stillborn, birth weight, survival to weaning, weaning weight), and effects on the progeny (body weight gain, weight of thymus, spleen and liver at termination, haematology [red and white blood cell counts, packed cell volume, mean corpuscular volume, haemoglobin], and immunocompetence [cell-mediated immunity, humoral immunity, number and phagocytic activity of peritoneal macrophages] at termination).				
Results:	No maternal toxicity. Litter size decreased ($p \leq 0.05$) and the number of stillborn increased ($p \leq 0.05$) at 500 mg/l.				
2,4-DCP (purity 99%)					
mouse CD-1 ICR m, f adult	0 (c)-0 (v-c)-200-600-2,000 mg/l drinking water (10% Emulphor), equal to 0-0-40-115-385 mg/kg/bw/day (males) or 0-0-50-145-490 mg/kg bw/day (females) (10 animals of each sex/group)	3-mo		≥ 385	Borzelleca et al., 1985b,c
Remarks:	After 90 days of exposure, dosing was continued throughout mating and gestation; 18 days after mating the animals were sacrificed.				
Parameters:	Fertility index, total number of implants, resorptions and live pups, and weight of individual pups.				
Results:	No statistically significant differences compared with the vehicle (Emulphor) control.				
2,4-DCP (purity 99%)					
rat Sprague-Dawley f age 3 w	0-3-30-300 mg/l drinking water, equivalent to 0-0.3-3-30 mg/kg bw/day (10-13 animals/group)	≤ 6-mo (prenatal exposure or pre- and postnatal exposure of progeny)	3**	0.3**	Exon et al., 1984; Exon & Koller, 1985
Remarks:	Prenatal only exposure groups: Dams were exposed from 3 w of age through gestation (bred at 90 d) and parturation. After parturation, dams were placed on control drinking water. Randomly selected pups from each group were weaned at 3 w of age and placed on control drinking water untill termination at 6 w of age.				

(to be continued)

Table 1.3 Reproductive toxicity studies with chlorophenols - (long-term) oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4-DCP (purity 99%) (continued)					
	Pre- and postnatal exposure groups: Exposure of dams was extended through lactation. Ten randomly selected pups from each group were weaned at 3 w of age and continued on treatment for 10-15 weeks. The design and results of this study have been reported in different publications which contain some inconsistencies. The results presented in this table have been derived from all data available.				
	Parameters: Maternal toxicity, reproductive performance (conception, litter size [live and stillborn], number of stillborn, birth weight, survival to weaning, weaning weight) and effects on the progeny (body weight gain, weights of thymus, spleen and liver at termination, histopathological changes in these organs, haematology [red and white blood cell cunts, packed cell volume, mean corpuscular volume, haemoglobin] and immunocompetence [cell-mediated immunity, humoral immunity, number and phagocytic activity of macrophages] at termination).				
	Results: According to Exon et al. (1984), no chemical-related effects were observed in the dams (no further details reported). Litter size was significantly ($p \leq 0.10$) reduced at 300 mg/l. Prenatal only exposure resulted in a significantly ($p \leq 0.05$) increase in spleen weight at 300 mg/l. Pre- and postnatal exposure resulted in a significantly ($p \leq 0.05$) decreased DTH-response (delayed-type hypersensitivity, used to measure cell-mediated immunity) at 30 and 300 mg/l ($p \leq 0.05$) and in an increase in serum antibody production to keyhole limpet hemocyanin which was significant ($p \leq 0.05$) at 300 mg/l. In addition, liver and spleen weight were significantly ($p \leq 0.05$) increased at 300 mg/l.				
2,4,6-TCP ("purified"; purity > 99%)					
rat Long-Evans f age 11 w	0-100-500-1,000 mg/kg bw/day by gavage (40 animals at 0 and 1,000; 20 animals at 100 and 500)	5-w	1,000	500	Blackburn et al., 1986
	Remarks: Animals were treated 5 days/week for two weeks prior to mating and then 7 days/weeks through day 21 of gestation. Litters were culled to 4 males and 4 females on day 4 postpartum and to two males and 2 females at weaning.				
	Parameters: Maternal toxicity (mortality, body weight), reproductive performance (date of delivery, conception, litter size, survival of pups at day 4 postpartum, body weight by sex of pups from day 1 through 42 postpartum) and vaginal patency of offspring.				
	Results: Treatment-related mortality (3/40) and decreased body weight gain of dams ($p \leq 0.05$), prior and during gestation, at 1,000 mg/kg bw/day (5/30 and 21/40 animals exposed to 500 and 1,000 mg/kg bw/day died due to intubation errors, caused by a marked increase in resistance to treatment). Body weights of male and female pups were reduced ($p \leq 0.05$) at day 1 postpartum, at 500 and 1,000 mg/kg bw/day, but this effect was not statistically significant after correcting for litter size. Subsequent weight gain up to day 42 postpartum was not affected.				

(to be continued)

Table 1.3 Reproductive toxicity studies with chlorophenols - (long-term) oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4,6-T3CP ("purified"; purity > 99%)					
rat Long-Evans m age 14 w	0-100-500-1,000 mg/kg bw/day by gavage (15 animals/group)	11-w	1,000	500	Blackburn et al., 1986
Remarks: Following treatment, control and high-dosed males were mated to untreated females. Females were sacrificed on day 18 of gestation.					
Parameters: Paternal toxicity (mortality, body and organ weights), reproductive performance (copulatory behaviour; sperm count, motility and morphology, number of litters, number of viable foetusses per litter, postimplantation loss, sex ration, foetal body weights male and females) and plasma testosterone.					
Results: Eight animals at 1,000 mg/kg bw/day died. All other parameters studied were not affected at any concentration tested.					
2,4,6-T3CP (purity 98%)					
rat Sprague Dawley f age 3 w	0-3-30-300 mg/l drinking water, equivalent to 0-0.3-3-30 mg/kg bw/day (12-14 animals/group)	± 6-mo pre- and postnatal exposure of progeny	3**	0.3**	Exon & Koller, 1985
Remarks: Dams were exposed from 3 w of age through gestation (bred at 90 d) and lactation. Ten randomly selected pups from each group were weaned at 3 w of age and continued on treatment for 12 weeks.					
Parameters: Reproductive performance (conception, litter size [live and stillborn], number of stillborn, birth weights, survival to weaning, weaning weight) and effects on the progeny (body weight gain, weight of thymus, spleen and liver, haematology [red and white blood cell counts, packed cell volume, mean corpuscular vlume, haemoglobin], and immunocompetence [cell-mediated immunity, humoral immunity, number and phagocytic activity of peritoneal macrophages] at termination.					
Results: Litter size was significantly ($p \leq 0.10$) decreased at 300 mg/l. Liver weight was significantly ($p \leq 0.05$) increased at 30 and 300 mg/l. In addition, spleen weight was increased significantly ($p \leq 0.05$) at 300 mg/l.					

(to be continued)

Table 1.3 Reproductive toxicity studies with chlorophenols - (long-term) oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("Dowicide EC-7", purity 90%; 10% T4CP; < 1 ppm OCDF, 2 ppm HpCDF, 3 ppm HxCDF, 15 ppm OCDD, 7 ppm HpCDD, 1 ppm HxCDD, < 0.05 ppm 2,3,7,8-TCDD)					
rat Sprague-Dawley m,f	0-3-30 mg/kg bw/day; dietary exposure (in feed) (10 males and 20 females/group)	3.5-mo (f) 5.5-mo (m) (pre- and postnatal exposure of progeny	30	3	Schwetz et al., 1978

Remarks: Females were exposed from 62 days prior to mating through gestation and lactation. Parent males were exposed for another two months. The dose levels in feed are not reported.

Parameters: Maternal and paternal toxicity (necropsy, body weight gain), reproductive performance (pregnancy, litter size, number of liveborn, birth weight, neonatal body weight through weaning, survival through weaning), and developmental effects (skeletal and soft tissue abnormalities).

Results: At 30 mg/kg bw/day, body weight gain of adult females, the number of liveborn pups, birth weight, neonatal body weight through weaning, and neonatal survival were reduced ($p \leq 0.05$). In addition, there was a significantly ("p" not reported) increased number of litters at this dose level which showed variations in the development of skeletal structures, namely lumbar spurs and vertebrae with unfused centra. It is not reported whether these variations were found also in control animals in this study or not, but in another study with this strain of rats these variations occured in all groups, including the control group (Schwetz et al., 1974b).

PCP ("technical-grade", purity 85%; 7% 2,3,4,6-T4CP; 400 ppm OCDD, 8 ppm HxCDD)					
rat Sprague-Dawley f age 3 w	0-5-50-500 mg/kg feed, equivalent to 0-0.25-2.5-25 mg/kg bw/day (12-14 animals/group	± 5-mo (pre- and postnatal exposure of progeny)	0.25*	.	Exon & Koller, 1982, 1983a,b

Remarks: Dams were exposed from 3 w of age through gestation (bred at 90 d) and lactation. Eight randomly selected pups from each group were weaned at 3 w of age and continued on treatment for 10 weeks. The design and results of this study have been reported in different publications which contain some inconsistencies. The results presented in this table have been derived from all data available.

Parameters: Maternal toxicity (body weight gain prior to breeding), reproductive performance (conception, litter size [live and stillborn], number of stillborn, birth weight, survival to weaning, weaning weight), and effects on the progeny (haematology [red and white blood cell counts, packed cell volume, mean corpuscular volume, haemoglobin] at weaning, and immunocompetence [cell-mediated immunity, humoral immunity, number and phagocytic activity of peritoneal macrophages] at termination).

(to be continued)

Table 1.3 Reproductive toxicity studies with chlorophenols - (long-term) oral exposure (continued)

Animal species	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical-grade", purity 85%; 7% 2,3,4,6-T4CP; 400 ppm OCDD, 8 ppm HxCDD) (continued)					
Results:	No maternal toxicity. Litter size was significantly ($p \leq 0.10$) decreased at 500 mg/kg. At all dose levels, both the DTH-response (delayed-type hypersensitivity, used to measure cell-mediated immunity) and the serum BSA antibody concentrations (used to measure humoral immunity) were significantly decreased, at $p \leq 0.03$ and $p \leq 0.0001$, respectively. In addition, the number and phagocytic activity of peritoneal macrophages were significantly ($p \leq 0.05$) increased at 50 and 500 mg/kg. Weaning weights of males, and, especially, females were generally decreased at all dose levels; a dose-relationship was not evident. (In separate experiments, liver weights were significantly increased in treated groups (no further data reported)				
PCP ("highly purified", purity > 99%; 1,25 ppb OCDD; no TCDD or TCDF)					
rat Sprague-Dawley m,f age 5 w	0-60-200-600 mg/kg feed, equal to 0-4-13-43 mg/kg bw/day for females (treatment groups: 20 animals/sex; control groups: 40 animals/sex)	6-mo	13	4	Welsh et al., 1987
Remarks:	Animals of both sexes were exposed for 181 days. Females were exposed from 5 w of age through gestation. Feed consumption was generally greater in the exposed groups.				
Parameters:	Maternal toxicity (mortality, feed consumption, weight gain during gestation, gross external and internal [major organs] abnormalities), reproductive performance (fertility, sex ratio, gravid uterus weight, number of corpora lutea, implantation efficiency, the number of dead and viable foetusses, birth weight), and developmental effects (skeletal and soft tissue abnormalities).				
Results:	Maternal weight gain (both with and without gravid uterus) was significantly ($p < 0.001$) reduced during gestation at 600 mg/kg feed. At this dose level, ringed eye was observed in 50% of the dams. At 200 mg/kg feed, the no. of dams with ≥ 2 resorptions was increased, and foetal body weight was reduced, both at $p < 0.05$. At 600 mg/kg feed, all but one foetusses were resorbed, due to early deaths. Treatment-related external and soft tissue foetal variations were not observed at any dose level. Misshapen centra of wavy ribs was the only skeletal variation that was significantly ($p < 0.05$) increased at 200 mg/kg feed: the incidence was 22/86 versus 14/167 in controls.				

LED: Lowest-effect-dose
NO(A)EL: No-observed-(adverse)-effect-level

* Feed study: standard "Conversion Factors" (mg/kg in feed : CF = mg/kg bw/day) of 7 and 20 have been used for mice and rats, respectively.

** Drinking water study: a standard "Conversion Factor" (mg/l in drinking water : CF = mg/kg bw/day) of 10 has been used for both mice and rats.

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2-MCP (purity 97%; impurities not reported)						
rat Sprague-Dawley f[#] age 3 w	C,T	0-5-50-500 mg/l drinking water, equivalent to 0-0.5-5-50 mg/kg bw/day	2-yr (pre- and postnatal exposure of progeny)	50[**]	5[**]	Exon & Koller, 1982, 1983a,b, 1985
2,4-DCP (purity > 99%)						
mouse B6C3F1 m,f age 9 w	T	0-2,500-5,000-10,000-20,000-40,000 mg/kg feed, equivalent to 0-350-700-1,400-2,800-5,600 mg/kg bw/day (10 animals of each sex/group)	3-mo	1,400[*]	@700[*]	NTP, 1989a

Remarks: [#]Dams (12-14/group) were exposed from weaning through gestation (bred at day 90 d) and lactation. The progeny (24-28 animals of each sex/group) from each treatment regimen was continued on treatment from 3 w of age (weaning) untill tumour development, death or termination of the experiment at 24 months.

Parameters: Reproductive performance and effects on the progeny (body and organ weights, haematology, immunocompetence and carcinogenicity). Only moribund and tumour bearing animals were examined microscopically.

Result: Carcinogenicity: **Negative in both sexes** (no compound-related effect on tumour incidence, latency or type of tumours in the progeny).
Toxicity: Of the haematological parameters studied, red blood cell count, packed cell volume, and haemoglobin content were significantly ($p \leq 0.10$) increased at 500 mg/l, in the second year of the study.
Effects on the other parameters mentioned are discussed in the section on reproductive toxicity (1.2.2 and table 1.3). The LED and NO(A)EL have been derived from all parameters studied.

Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined.

Results: All animals exposed to 40,000 mg/kg feed died. At 20,000 mg/kg feed, final body weight (males) or body weight throughout most of the study (females) was reduced 10%-15%. Growth was not affected at ≤ 10,000 mg/kg feed. Feed consumption was reduced (20%-70%) in all groups exposed to ≥ 10,000 mg/kg feed.
Syncitial alteration of hepatocytes were observed in all animals exposed to ≥ 10,000 mg/kg feed. A dose-related increase in the incidence of hepatocellular necrosis was seen in males (0/10, 4/10, 4/10, 6/10, 10/10 at 0, 2,500, 5,000, 10,000 and 20,000 mg/kg feed, respectively); no further details on this effect reported (NTP considered the severity of this lesion to be "minimal" in the three lowest dose groups. Furthermore, this lesion was also found in 3/10 control females).

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4-DCP (purity 99%)						
mouse CD-1 ICR m, f age 6 w	T	0 (c)-0 (v-c)-200-600-2,000 mg/l drinking water (10% Emulphor), equal to 0-0-40-115-385 mg/kg/bw/day (males) or 0-0-50-145-490 mg/kg bw/day (females) (20 animals of each sex/group)	3-mo	.	≥ 385	Borzelleca et al.,'85b,c
Parameters: Mortality, water consumption, terminal body and organ weights, haematology, clinical chemistry, hepatic microsomal mixed function oxidase system (components and component activities).						
Results: The only effects observed, were a dose-related increase in leucocyte count ($p < 0.05$ at 2,000 mg/l in males only) and an increase ($p < 0.05$) in ALP activity in males at 2,000 mg/l. These effects are not considered to be toxicologically significant alterations (In the deionized water control a number of parameters (e.g. organ weights) was significantly different compared to those in the vehicle-control, showing that the vehicle itself was not without effect)						
2,4-DCP (purity not reported)						
mouse ICR m	T	0-200-500-1,000-2,000 mg/kg feed, equal to 0-20-45-100-230 mg/kg/bw/day (7 animals per group)	6-mo	230	100	Kobayashi et al., '72
Parameters: Feed consumption, body weight gain, weights and histopathology of major organs (liver, kidneys, spleen, heart), haematology (red and white blood cell counts) and clinical chemistry (serum glutamate oxaloacetate transaminase activity; serum glutamate pyruvate transaminase).						
Results: At the highest dose level, one or two animals showed minor histological changes in the liver (small round cell infiltration, swelling or unequal size of hepatocytes, dark cell)						
2,4-DCP (purity > 99%)						
mouse B6C3F1 m,f age 8 w	C,T	0-5,000-10,000 mg/kg feed, equal to 0-800-1,300 mg/kg bw/day (m) or 0-430-820 mg/kg bw/day (f) (50 animal of each sex/group)	2-yr	800 (m) 820 (f)	. (m) 430 (f)	NTP, 1989a
Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).						
Results: Carcinogenicity: **Negative in both sexes** (no compound-related increases in malignant or benign neoplasms). Toxicity: At 10,000 mg/kg, body weight of females was reduced progressively; from week 25 and onwards, the reduction was ≥ 10% (feed consumption was reduced 15% throughout the study). Body weights of the other dosed groups were within 10% of that of controls, although feed consumption of males exposed to 10,000 mg/kg feed was reduced 22% throughout the study. A dose-related, significantly ($p < 0.001$) increased incidence of diffuse syncitial alteration of hepatocytes was observed in males (11/50 in controls; 33/49 and 42/48 at 5,000 and 10,000 mg/kg feed, respectively). No other compound-related changes were found at histological examination.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4-DCP (purity > 99%)						
rat F344/N m,f age 7-w	T	0-2,500-5,000-10,000-20,000-40,000 mg/kg feed, equivalent to 0-125-250-500-1,000-2,000 mg/kg bw/day (10 animals of each sex/group)	3-mo	1,000* 500*	500* (m) 250* (f)	NTP, 1989a
Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined.						
Results: Final weight relative to controls was reduced 10%-40% in all groups at ≥ 20,000 mg/kg feed (feed consumption by these groups was reduced 10%-30%); Final weight relative to controls were within 5% in all groups exposed to ≤ 10.000 mg/kg feed. Bones marrow atrophy (depletion of both erythroid and myelocytic elements) was observed in all animals exposed to ≥ 20,000 mg/kg feed and in 6/10 females at 10,000 mg/kg.						
2,4-DCP (purity > 99%)						
rat F344/N m age 7 w	C,T	0-5,000-10,000 mg/kg feed, equal to 0-210-440 mg/kg/bw/day (50 males/group)	2-yr	440	@210	NTP, 1989a
Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).						
Results: Carcinogenicity: **Negative** (no compound-related increases in malignant or benign neoplasms) Toxicity: At 10,000 mg/kg, body weight relative to controls was reduced (5%-10%) consistenly throughout the study (feed consumption reduced 5%). The incidence of multifocal degeneration of respiratory epithelium of the nose was dose-related increased (25/45, 38/48 [p < 0.05] and 42/46 [p = 0.001], respectively). No other compound-related pathological changes were found at histological examination.						
2,4-DCP (purity > 99%)						
rat F344/N f age 7 w	C,T	0-2,500-5000 mg/kg feed, equal to 0-120-250 mg/kg bw/day (50 females/group)	2-yr	250	120	NTP, 1989a
Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).						
Results: Carcinogenicity: **Negative** (no compound-related increases in malignant or benign neoplasm) Toxicity: At 5,000 mg/kg feed, body weight relative to controls was reduced (5%-10%) consistently from week 10 and onwards (feed consumption reduced 6%). No compound-related pathological changes were found at histological examination.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4-DCP (purity 99%)						
rat Sprague-Dawley f[#] age 3 w	C,T	0-3-30-300 mg/l drinking water, equivalent to 0-0.3-3-30 mg/kg bw/day	2-yr (pre- and postnatal exposure of progeny)	3**	0.3**	Exon et al., 1984; Exon & Koller, 1985

Remarks: [#]Dams (12-14/group) were exposed from weaning through gestation (bred at day 90 d) and lactation. The progeny (22-29 animals of each sex/group) from each treatment regimen was continued on treatment from 3 w of age (weaning) untill tumour development, death or termination of the experiment at 24 months.

Parameters: Reproductive performance and effects on the progeny (body and organ weights, haematology, immonocompetence, and carcinogenicity). Only moribund and tumour bearing animals were examined microscopically.

Results: Carcinogenicity: **Negative in both sexes** (no compound-related effect on tumour incidence, latency or type of tumours in the progeny).
Toxicity: Of the haemastological parameters studied, red blood cell count and haemoglobin content were significantly ($p \leq 0.05$) increased at 300 mg/l, in the second year of the study.
Effects on the other parameters mentioned are discussed in the section on reproductive toxicity (1.2.2 and table 1.3). The LED and NO(A)EL have been derived from all parameters studied.

2,4,5-T3CP (purity > 99%)						
rat Wistar m,f age 7 w	T	0-100-300-1,000-3,000-10,000 mg/kg feed, equivalent to 0-5-15-50-150-500 mg/kg bw/day (10 animals of each sex/group)	3-mo	150*	50*	McCollister et al., 1961

Remarks: Histopathology very briefly reported. The authors used a conversion factor of 10, without further information.

Parameters: Mortality, feed consumption, body weight (growth), relative weight of the major organs, gross and microscopic examination of the major organs, and haematology.

Results: At 10,000 mg/kg feed, growth was reduced (statistically significant in females only; $p < 0.05$).
At concentrations ≥ 3,000 mg/kg feed, a diuretic effect was observed, and, in addition, pathological changes in kidneys ("moderate degenerative changes in the epithelium lining of the convoluted tubules and early proliferation of the interstitial tissue") and liver ("mild centrilobular degenerative changes characterized by cloudy swelling and an occasional area of focal necrosis"). These changes were considered to be of a mild, reversible nature. However, the severity of these lesions was dose-related.
(The investigators mention a Conversion Factor of 10, without further information)

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4,6-T3CP ("Omal", "Dowicide 2S", purity 96%-97%; 17 minor contaminants [not specified]; chlorinated dibenzo-p-dioxins not determined)						
mouse B6C3F1 m age 6 w	C,T	0-5,000-10,000 mg/kg feed, equivalent to 0-700-1,400 mg/kg bw/day (50 dosed and 20 control animals)	2-yr	700*	.	NCI,1979

Parameters: Mortality, body weight, palpation for masses, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).

Results: Carcinogenicity: **Positive** (dose-related increase in malignant and benign neoplasms). Dose-related increase in hepatocellular carcinomas (**5%** [1/20] - **20%** [10/49] - **15%** [7/47]; $p \leq 0.001$ and $p < 0.001$) and hepatocellular adenomas (**15%** [3/20] - **45%** [22/49] - **68%** [39/47]; $p = 0.001$ and $p < 0.001$). The historical incidence of hepatocellular adenomas and carcinomas in male B6C3F1 mice is 30% (99/323).
Toxicity: Body weight was dose-related decreased throughout the study. In dosed animals, hepatocellular damage (ranging from individual liver cell abnormalities, through focal areas of cellular alteration, to focal and nodular areas of hyperplasia) was commonly present.

2,4,6-T3CP ("Omal", "Dowicide 2S", purity 96%-97%; 17 minor contaminants [not specified; chlorinated dibenzo-p-dioxins not determined)						
mouse B6C3F1 f age 6 w	C,T	0-10,000-20,000 mg/kg feed for 38 weeks, and 0-2,500-5,000 mg/kg feed thereafter; Time-weighted average dose level equivalent to 0-750-1,500 mg/kg bw/day	2-yr	750*	.	NCI, 1979

Parameters: Mortality, body weight, palpation for masses, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).

Results: Carcinogenity: **Positive** (dose-related increase in malignant and benign neoplasms). Dose-related increase in hepatocellular carcinomas (**0%** [0/20] - **0%** [0/50] - **15%** [7/48]; n.s.) and hepatocellular adenomas (**5%** [1/20] - **24%** [12/50] - **35%** [17/48]; n.s. and $p < 0.001$, respectively. The historical incidence of hepatocellular adenomas and carcinomas in female B6C3F1 mice is 4% (14/324).
Toxicity: Body weight was dose-related decreased throughout the study. In dosed animals, hepatocellular damage (ranging from individual liver cell abnormalities, through focal areas of cellular alteration, to focal and nodular areas of hyperplasia) was commonly present.

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
2,4,6-T3CP ("Omal", "Dowicide 2S", purity 96%-97%; 17 minor contaminants[not specified; chlorinated dibenzo-p-dioxins not determined)						
rat F344 m, f	C,T	0-5,000-10,000 mg/kg feed, equivalent to 0-250-500 mg/kg bw/day	2-yr	250*	.	NCI, 1979
Parameters: Mortality, body weight, palpation for masses, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).						
Results: Carcinogenicity: **Positive in males; negative in females.** In males there was a dose-related increase in (monocytic) leukemias (**15%** [3/20], - **46%** [23/50] - **56%** [29/50]; p = 0.01 and p = 0.002). The historical incidence in male F344 rats is 4% (11/255). In females there also was an increase in (monocytic) leukemias (**15%** [3/20] - **22%** [11/50] - **20%** [10/50], but this increase was not significant at $p \leq 0.05$. Toxicity: Body weight was dose-related decreased throughout the study. In all groups, the incidences of non-neoplastic lesions were considered to be within normal limits.						
PCP ("pure", purity > 99%; impurities not reported)						
mouse C57Bl/6 (B6) m 8 w	T	0-50-500 mg/kg feed, equivalent to 0-7-70 mg/kg bw/day	3-mo	7	.	Kerkvliet et al., 1982
Parameters: Body weight, histopathology major organ (liver, kidneys, spleen, adrenal tissues) and immunocompetence (susceptibility to low-dose syngeneic tumour transplant (3-methylchlolanthrene-induced sarcoma of B6 origin), susceptibility to primary Moloney sarcoma virus -(MSV)-induced tumour growth and secundary challenge with MSV-transformed sarcoma cells, susceptibility to encephalomyocarditis virus infection, T-cell cytolytic activity, and macrophage phagocytosis activity (the latter two effects measured in vitro).						
Results: Histopathological examinations showed dose-related liver lesions (mild to marked swelling of hepatocytes, accompanied by nuclear swelling and vacuolization; eosinophilic inclusion bodies within nuclear vacuoles). Mild to moderate multifocal necrosis was observed only at 500 mg/kg feed. Treatment did not effect the incidences of the tumour types inducted by any challenge. However, in surviving animals that were resistent to both the MSV and MSB challenge, a dose-related increase in gross tumours in spleen (0/13-2/9-4/9) was observed.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical-grade", purity 86%; impurities not reported)						
mouse C57B1/6 (B6) m 8 w	T	0-50-500 mg/kg feed, equivalent to 0-7-70 mg/kg bw/day	3-mo	7	.	Kerkvliet et al., 1982

Parameters: Body weight, histopathology major organ (liver, kidneys, spleen, adrenal tissues) and immunocompetence (susceptibility to low-dose syngeneic tumour transplant (3-methylchlolanthrene-induced sarcoma of B6 origin), susceptibility to primary Moloney sarcoma virus -(MSV)-induced tumour growth and secundary challenge with MSV-transformed sarcoma cells (MSB), susceptibility to encephalomyocarditis virus infection, T-cell cytolytic activity, and macrophage phagocytosis activity (the latter two effects measured in vitro).

Results: Histopathological examinations showed dose-related liver lesions (mild to marked swelling of hepatocytes, accompanied by nuclear swelling and vacuolization; eosinophilic inclusion bodies within nuclear vacuoles). Mild to moderate multifocal necrosis was observed only at 500 mg/kg feed. Treatment resulted in a dose-related ($p < 0.005$) enhancement of susceptibility to tumour induction, regardless of the challenge used. Additionally, in surviving animals that were resistent to both the MSV and MSB challenge, an increase in gross tumours in spleen (3/6 versus 0/13) was observed at 50 mg/kg feed; this type of tumour was not observed in the 3 surviving animals at 500 mg/kg. T-cell cytolytic activity was reduced and macrophage phagocytic acticity increased (both at $p < 0.05$) at 500 mg/kg feed.

PCP ("pure", purity 98.6%; 1.4% T4CP; 2,200 ppm heptachlorohydroxydibenzofuran, 1,100 ppm hexachlorohydroxydibenzofuran, 2,100 ppm nonachlorohydroxydiphenyl ether, 900 ppm octachlorohydroxydiphenyl ether, 100 ppm heptachlorohydroxydiphenyl ether, < 1 ppm OCDD, < 1 ppm HxCDD, < 1 ppm TCDD)						
mouse B6C3F1 m,f 7-9 w	T	0-200-500-1,500 mg/kg feed, equivalent to 0-28-70-210 mg/kg bw/day (treatment groups: 25 m and 10 f; control group: 48 m and 10 f)	6-mo	28*	.	NTP, 1989b

Parameters: Mortality, feed consumption, body weight, organ weights (liver, spleen, thymus), gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined). Supplemental studies included haematology, clinical chemistry, urinanalysis, immunologic analysis, aryl hydrocarbon hydroxylase, oxidative phosporylation, cytochrome P450, porphyrins, and body temperature).

Results: At 1,500 mg/kg feed, final weight relative to controls was reduced (10%) in both sexes (feed consumption not affected). Compound-related effects were found in several tissues, especially in the liver. At all dose levels tested, liver weight was significantly increased in both sexes, and histopathological liver changes (necrosis, nuclear alteration, cytomegaly, pigmentation) were found in most animals examined (10/group).
For data on supplemental studies: see the text.

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("Dowicide EC-7", purity 91%; 9% T4CP; 0.2 ppm HpCDF, 0.1 ppm HxCDF, 0.7 ppm OCDD, 0.5 ppm HpCDD, 0.2 ppm HxCDD, < 0.04 ppm TCDD)						
mouse B6C3F1 m,f 7-9 w	T	0-200-600-1,200 mg/kg feed, equivalent to 0-28-85-170 mg/kg bw/day (treatment groups: 25 m and 10 f; control group: 48 m and 10 f)	6-mo	28*	.	NTP, 1989b
Parameters: Mortality, feed consumption, body weight, organ weights (liver, spleen, thymus), gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined). Supplemental studies included haematology, clinical chemistry, urinanalysis, immunologic analysis, aryl hydrocarbon hydroxylase, oxidative phosporylation, cytochrome P450, porphyrins, and body temperature).						
Results: At 1,200 mg/kg feed, final body weight relative to controls was decreased (> 10%) in both sexes. Compound-related effects were found in several tissues, especially in the liver. At all dose levels tested, liver weight of females was significantly increased; that of males was increased only at the highest dose level tested. At all dose levels, histopathological liver changes (necrosis, nuclear alteration, cytomegaly, pigmentation) were found in most animals examined (10/group). For data on supplemental studies: see the text.						
PCP ("DP-2", purity 92%; 7% T4CP, 2.2% nona-, 1.4% octa- and 0.05% heptachlorohydroxydiphenyl ether; 3,100 ppm hepta- and 700 ppm hexachlorohydroxydibenzofuran; 320 ppm OCDF, 170 ppm HpCDF, 13 ppm HxCDF, 175 ppm OCDD, 30 ppm HpCDD, 5,900 ppm HxCDD; TCDD not quantitated)						
mouse B6C3F1 m,f 7-9 w	T	0-200-600-1,200 mg/kg feed, equivalent to 0-28-85-170 mg/kg bw/day (treatment groups: 25 m and 10 f; control group: 48 m and 10 f)	6-mo	28*	.	NTP, 1989b
Parameters: Mortality, feed consumption, body weight, organ weights (liver, spleen, thymus), gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined). Supplemental studies included haematology, clinical chemistry, urinanalysis, immunologic analysis, aryl hydrocarbon hydroxylase, oxidative phosporylation, cytochrome P450, porphyrins, and body temperature).						
Results: Compound-related effects were found in several tissues, especially in the liver. At all dose levels tested, liver weight was significantly increased, with exception of that of males fed 200 mg/kg feed. In addition, at all dose levels histopathological liver changes (necrosis, nuclear alteration, cytomegaly, pigmentation) were found in most animals examined (10/group). For data on supplemental studies: see the text.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical grade", purity 90%; 3.8% T4CP; 3.6% nona-, 1.9% octa- and 0.1% heptachlorohydroxydiphenyl ether; 0.5% hepta- and 0.2% hexachlorohydroxydibenzofuran; 45 ppm OCDF, 90 ppm HpCDF, 10 ppm HxCDF, 1.4 ppm PeCDF, 1,390 ppm OCDD, 300 ppm HpCDD, 10 ppm HxCDD, TCDD not quantitated)						
mouse B6C3F1 m,f 7-9 w	T	0-200-600-1,800 mg/kg feed, equivalent to 0-28-85-255 mg/kg bw/day (treatment groups: 25 m and 10 f; control group: 48 m and 10 f)	6-mo	28*	.	NTP, 1989b

Parameters: Mortality, feed consumption, body weight, organ weights (liver, spleen, thymus), gross pathology at necropsy, histopathology (very comprehensive with regard to the number of different tissues examined). Supplemental studies included haematology, clinical chemistry, urinanalysis, immunologic analysis, aryl hydrocarbon hydroxylase, oxidative phosporylation, cytochrome P450, porphyrins, and body temperature).

Results: All animals that were fed 1,800 mg/kg feed died. Compound-related effects were found in several tissues, especially in the liver. At all dose levels tested, liver weight was significantly increased in both sexes, and at all dose levels histopathological liver changes (necrosis, nuclear alteration, cytomegaly, pigmentation) were found in most animals examined (10/group).
For data on supplemental studies: see the text.

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("Dowicide EC-7", purity 91%; 9% T4CP; 0.2 ppm HpCDF, 0.1 ppm HxCDF, 0.7 ppm OCDD, 0.5 ppm HpCDD, 0.2 ppm HxCDD, < 0.04 ppm TCDD)						
mouse B6C3F1 m,f 9 w	C,T	0-100-200-600 mg/kg feed, equal to 0-17-35-116 (50 dosed and 35 controls of each sex)	2-yr	17	.	NTP, 1989b

Remarks: Dose levels based on feed consumption were very similar for males and females.

Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).

Results: Carcinogenicity: **Positive in both sexes** (dose-related increase in both malignant and benign neoplasms)

In males there was a dose-related increase in hepatocellular carcinomas (**3%** [1/35] - **15%** [7/48] - **15%** [7/48] - **18%** [9/49]; p = 0.07, 0.07 and 0.03), hepatocellular adenomas (**9%** [5/35] - **27%** [13/48] **35%** [17/48] - **65%** [32/49]; p = 0.13, 0.03 and < 0.001), and adrenal medullary pheochromocytomas (**3%** [1/34] - **8%** [4/48] - **44%** [21/48] - **92%** [45/49]; p = 0.3, < 0.001 and 0.001); in the control and high-dosed group, 1 and 3 pheochromocytomas were regarded to be malignant, respectively. The historical incidences of hepatocellular carcinomas, hepatocellular adenomas and adrenal pheochromocytomas in male B6C3F1 mice are 19% (8%-30%), 13% (0%-44%) and 1.5% (0%-8%), respectively. In females there was a dose-related increase in hepatocellular adenomas (**3%** [1/34] - **6%** [3/50] - **12%** [6/49] - **62%** [30/48]; p = 0.46, 0.13 and < 0.001), adrenal medullary pheochromocytomas (**0%** [0/35] - **4%** [2/49] - **4%** [2/46] - **78%** [38/49]; p = 0.38, 0.32 and < 0.001), and in hemangiosarcomas (**0%** [0/35] **2%** [1/50] - **6%** [3/50] - **16%** [8/49]; p = 0.6, 0.2 and 0.01) in spleen and liver. Only one of the pheochromocytomas (low-dose group) was judged to be malignant. The historical incidences of hepatocellular adenomas and hemangiosarcomas in female B6C3F1 mice are 5% (0%-18%) and 1.6% (0%-8%), respectively. That of adrenal pheochromocytomas in female mice is not reported.

Toxicity: At 600 mg/kg, body weight of females was reduced consistently and progressively from week 36 and onwards, resulting in 20% reduction towards the end of the study, while feed consumption was not affected adversely. High (≥ 40%) to very high incidences of histopathological liver changes (acute diffuse necrosis, diffuse chronic active inflammation, diffuse cytomegaly, multifocal pigmentation) were observed in all dose groups; these changes were not observed in any of the controls. A very high incidence (≥ 65%) of bile duct hyperplasia (an increase in small bile ductules) was observed only at 600 mg/kg feed, in both males and females.

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical grade", purity 90%; 3.8% T4CP; 3.6% nona-, 1.9% octa- and 0.1% heptachlorohydroxydiphenyl ether; 0.5% hepta- and 0.2% hexachlorohydroxydibenzofuran; 45 ppm OCDF, 90 ppm HpCDF, 10 ppm HxCDF, 1.4 ppm PeCDF, 1,390 ppm OCDD, 300 ppm HpCDD, 10 ppm HxCDD, TCDD not quantitated)						
mouse B6C3F1 m,f 9 w	C,T	0-100-200 mg/kg feed, equal to 0-17-35 mg/kg bw/day (50 dosed and 35 controls of each sex)	2-yr	17	.	NTP, 1989b
Remarks: Dose levels based on feed consumption were equal for males and females.						
Parameters: Mortality, feed consumption, body weight, gross pathology at necropsy, and histopathology (very comprehensive with regard to the number of different tissues examined).						
Results: Carcinogenicity: **Positive in both sexes** (dose-related increase in [i] both malignant and benign neoplasms in males, and [ii] malignant neoplasms in females). In males there was a dose-related increase in hepatocellular carcinomas (**6%** [2/32] - **21%** [10/47] - **25%** [12/48]; p = 0.06 and 0.03), hepatocellular adenomas (**16%** [5/32] - **43%** [20/47] - **69%** [33/48]; p = 0.01 and < 0.001), and benign adrenal medullary pheochromocytomas (**0%** [0/31] - **22%** [10/45] - **51%** 23/45]; p = 0.003 and < 0.001). The historical incidences of hepatocellular carcinomas, hepatocellular adenomas and adrenal pheochromocytomas in male B6C3F1 mice are 19% (8%-30%), 13% (0%-44%) and 1.5% (0%-8%), respectively. In females there was a dose-related increase in hemangiosarcomas (**0%** [0/35] - **6%** [3/50] - **12%** [6/50]; p = 0.2 and 0.04); all hemangiosarcomas were observed in the spleen and liver. The historical incidence of these neoplasms in female B6C3F1 mice is 1.6% (0%-8%). Toxicity: Very high incidences (≥ 70%) of histopathological liver changes (acute diffuse necrosis, diffuse chronic active inflammation, diffuse cytomegaly, multifocal pigmentation) were found in all dose groups; these changes were not observed in any of the controls. A high incidence (≥ 50%) of bile duct hyperplasia (increase in small bile ductules) was observed in dosed males, but not in dosed females.						
PCP ("pure"; purity not reported; no detectable concentrations of any PCDD)						
rat sprague-Dawley	T	0-3-10-30 mg.kg bw/day, administered in feed	3-m	10	3	Johnson et al., 1973
Remarks: The number, age and sex of test animals in not reported.						
Parameters: Feed consumption, body and organ (liver, kidneys) weights, gross pathology at necropsy, histopathology, haematology, urinanalysis and clinical chemistry						
Results: Terminal weights of liver and kidneys were increased at 30 mg/kg bw/day; that of liver was also increased at 10 mg/kg bw/day.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("improved"; purity 88%-93%; 7%-12% T4CP; 26 ppm OCDD, 1 ppm HxCDD, < 0.05 ppm 2,3,7,8-TCDD)						
rat sprague-Dawley	T	0-3-10-30 mg.kg bw/day, administered in feed	3-m	10	3	Johnson et al., 1973
Remarks: The number, age and sex of test animals in not reported.						
Parameters: Feed consumption, body and organ (liver, kidneys) weights, gross pathology at necropsy, histopathology, haematology, urinanalysis and clinical chemistry						
Results: Terminal weights of liver and kidneys were increased at 30 mg/kg bw/day; that of liver was also increased at 10 mg/kg bw/day.						
PCP ("technical-grade"; purity 85%-90%; 10%-15% T4CP; 2,000 ppm OCDD, 20 ppm HxCDD; < 0.05 ppm 2,3,7,8-TCDD)						
rat sprague-Dawley	T	0-3-10-30 mg.kg bw/day, administered in feed	3-m	3	.	Johnson et al., 1973
Remarks: The number, age and sex of test animals in not reported.						
Parameters: Feed consumption, body and organ (liver, kidneys) weights, gross pathology at necropsy, histopathology, haematology, urinanalysis and clinical chemistry						
Results: Terminal weights of liver and kidneys, and serum alkaline phosphatase were increased at all dose levels tested. Additionally, serum albumin was decreased at 10 and 30 mg/kg bw/day, and histopathological liver changes (minimal focal hepatocellular degeneration and necrosis) were observed and haematological parameters (erythrocyte count, haemoglobin content and packed cell volume) were decreased at 30 mg/kg bw/day.						
PCP (purity not reported; 200 ppm OCDD, 80 ppm pre-OCDD, TCDD not detectable; no data on other impurities)						
rat Wistar-SPD m,f weanling	T	0-25-50-200 mg/kg feed, equivalent to 0-1.25-2.5-10 mg/kg bw/day	3-mo	2.5*	1.25*	Knudsen et al., 1974
Parameters: Feed consumption, body and organ weights, histopathology, activity of microsomal enzymes (AH, APDM, Glu-6-P), haematology, clinical chemistry and urinanalysis.						
Results: Body weight gain of females was significantly ($p \leq 0.05$) reduced at 200 mg/kg; feed consumption was not affected. Liver weight of females was significantly increased at 50 and 200 mg/kg. Increased incidences of histopathological changes (both centrilobular vacuolisation in the liver of males and a lower number of calculi in corticomedullary junction of the kidneys in females) were observed at 50 and 200 mg/kg. In addition, haemoglobin content and the number of erythrocytes in the blood of males were significantly increased at both 50 and 200 mg/kg. Serum glucose (males), serum alkaline phosphatase activity (females) and the activity of the microsomal enzymes aniline hydroxylase and aminopyrine demethylase was significantly increased at 200 mg/kg.						

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("purified", purity > 99%; < 0.1 ppm of each group of isomers of dibenzo-p-dioxins and dibenzofurans)						
rat Sherman f age 4 w	T	0-20-100-500 mg/kg feed, equivalent to 0-1-5-25 mg/kg bw/day (6 animals/group)	8-mo	25*	5*	Goldstein et al., 1977

Parameters: Feed consumption, body weight, liver weight, hepatic drug-metabolizing enzymes (aryl hydrocarbon hydroxylase, glucuronyl transferase, cytochroom P450, N-demethylase, ALA synthethase), liver porphyria (fluorescence), microsomal heme, urinary porphyrins and their precursors (ALA, PBG).

Results: At 500 mg/kg feed, body weight was reduced ($p < 0.05$), although feed consumption was not reduced. In addition, hepatic glucuronyl transferase activity was increased 3-fold ($p < 0.05$) and several livers were totally dark or contained dark areas, at this dose level.

PCP ("purified", purity > 99%; < 0.1 ppm of each group of isomers of dibenzo-p-dioxins and dibenzofurans)						
rat Sherman m,f weanling	T	0-20-100-500 mg/kg feed, equivalent to 0-1-5-25 mg/kg bw/day (10 animals of each sex/group)	8-mo	25*	5*	Kimbrough & Linder, 1978

Remarks: The dose levels of 1, 5, and 25 mg/kg/bw/day calculated using a standard conversion factor of 20, are very similar to the dose levels based on feed consumption from day 80 to termination. At start, the latter dose levels were about 2 times higher.

Parameters: Mortality, feed consumption, body weight, weight of major organs (liver, kidneys, spleen, heart, brain, lungs, testes), histopathology (aforementioned organs and thyroid, parathoid, gastrointestinal tract, reproductive organs, gall bladder and adrenals)

Results: At 500 mg/kg feed, body weight gain was reduced in both sexes, but only statistically significant ($p < 0.05$) in males; feed consumption was similar to that by controls. In addition, microscopic examinations showed minor hepatocellular alterations in a number of animals (slightly brownish diffuse discoloration in females, slightly enlarged hepatocytes around central veins in both sexes, cytoplasmic eosinophilic inclusions in males, and a brown pigment in macrophages of females). (Weight of kidneys of males was significantly increased at all dose levels (3.0 g in the treated groups versus 2.6 g in control males), but microscopic findings were normal.

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("technical-grade", purity 85%; 3% 2,3,4,6-T4CP; 1,380 ppm OCDD, 520 ppm HpCDD, 8 ppm HxCDD, < 0.1 ppm PeCDD, < 0.1 ppm TCDD [2,3,7,8-TCDD not detected], 260 ppm OCDF, 400 ppm HpCDF, 90 ppm HxCDF, 40 ppm PeCDF and 4 ppm TCDF; chlorophenyl ethers were detected but not quantitated)						
rat Sherman f age 4 w	T	0-20-100-500 mg/kg feed equivalent to 0-1-5-25 mg/kg bw/day (6 animals/group)	8-mo	1*	.*	Goldstein et. al., 1977
PCP ("technical-grade", purity 85%; 3% 2,3,4,6-T4CP; 1,380 ppm OCDD, 520 ppm HpCDD, 8 ppm HxCDD, < 0.1 ppm PeCDD, < 0.1 TCDD [2,3,7,8-TCDD not detected], 260 ppm OCDF, 400 ppm HpCDF, 90 ppm, HxCDF, 40 ppm PeCDF and 4 ppm TCDF; chlorophenyl ethers were detected but not quantitated)						
rat Sherman m,f weanling	T	0-20-100-500 mg/kg feed, equivalent to 0-1-5-25 mg/kg bw/day (10 animals of each sex/group)	8-mo	1*	.	Kimbrough & Linder, 1978

Parameters: Feed consumption, body weight, liver weight, hepatic drug-metabolizing enzymes (aryl hydrocarbon hydroxylase, glucuronyl transferase, cytochroom P450, N-demethylase, ALA synthethase), liver porphyria (fluorescence), microsomal heme, urinary porphyrins and their precursors (ALA, PBG).

Results: At 500 mg/kg feed, body weight was reduced ($p < 0.05$), although feed consumption was not reduced. Dose-related increases in aryl hydrocarbon hydroxylase activity (3- to 7-fold) and glucuronyl transferase activity (15- to 43-fold) were observed, which were statistically significant ($p < 0.05$) at all dose levels tested. In addition, a significantly altered ratio of the 455/430 nm peaks of the ethylisocyanide difference spectrum of cytochrome P450 was observed at all dose levels, caused by a shift from 455 to 453 nm. Dose levels of 100 and 500 mg/kg feed resulted in an increase in liver weight, cytochrome P450 content, microsomal heme, and liver and urine porphyrins. At these dose levels, several livers were totally dark or contained dark areas.

Remarks: The dose levels of 1, 5, and 25 mg/kg/bw/day calculated using a standard conversion factor of 20, are very similar to the dose levels based on feed consumption from day 80 to termination. At start, the latter dose levels were about 2 times higher.

Parameters: Mortality, feed consumption, body weight, weight of major organs (liver, kidneys, spleen, heart, brain, lungs, testes), histopathology (liver, kidneys, spleen, heart, lungs and brain).

Results: At 500 mg/kg feed, body weight gain was significantly reduced and liver weight was increased, in both sexes ($p < 0.05$). Microscopic examinations of the organs showed a variety of morphological changes in the livers of most animals at 100 and, in particular, at 500 mg/kg feed; these changes included (enlarged hepatocytes, vacuolation of the cytoplasm, brown pigmentation in macrophages and Kupffer cells and fibrosis. Additional changes (hepatocytes with karyorrhectic or pyknotic nuclei, bile duct proliferation, hyaline bodies, increased mitotic figures) were found in females only, especially at 500 mg/kg feed; at this dose level, the outer surface of livers was irregular with pitted area of retraction. At 20 mg/kg feed, minor lesions were found in the liver (centrolobular hepatocytes were slightly enlarged and occasionally vacuolated in all males and in one female).

(to be continued)

Table 1.4 Semichronic and chronic toxicity and carcinogenicity studies with chlorophenols - oral exposure (continued)

Animal species	Study type	Exposure	Exposure time	Result mg/kg bw/day LED	Result mg/kg bw/day NO(A)EL	Reference
PCP ("Dowicide EC-7", purity 90%; 10% T4CP; < 1 ppm OCDF, 2 ppm HpCDF, 3 ppm HxCDF, 15 ppm OCDD, 7 ppm HpCDD, 1 ppm HxCDD, < 0.05 ppm 2,3,7,8-TCDD)						
rat Sprague-Dawley m,f	C,T	0-1-3-10-30 mg/kg bw/day, administered in feed (27 animals of each sex/group)	22-mo (m) 24-mo (f)	30 10	10 3	Schwetz et al., 1978

Remarks: Males were terminated after 22 months, because of high mortality among control and dosed animals. The levels in feed were not reported.

Parameters: Mortality, feed consumption, body weight, organ weight (liver, kidneys, heart, brain ,testes), gross pathology at necropsy, histopathology, haematology, clinical chemistry and urinalysis.

Results: Carcinogenicity: **Negative in both sexes** (no compound-related increases in malignant or benign neoplasms.

Toxicity: At 30 mg/kg bw/day, body weight of females was reduced significantly ("p" not reported) throughout the study, and serum alanine aminotransferase (ALAT) activity was significantly increased in both sexes, at termination. At 10 and 30 mg/kg bw/day, dark discoloration (caused by granular pigmentation) of liver and kidneys were observed in a number of females, especially at the highest dose level tested. Microscopic examinations showed liver pigmentation in 8/27 and 16/27 females at these dose levels, respectively. Pigmentation of kidneys was found in 7/27 and 19/27 females, respectively. Pigmentation was not observed in control animals, and in only 1/27 males (liver).

Study type: C = carcinogenicity; T = toxicity (non-carcinogenic effects)

LED: Lowest-effect-dose

NO(A)EL: No-observed-(adverse)-effect-level; @NO(A)EL: marginal NO(A)EL [the effect(s) found at this concentration are considered to be of minor biological significance]

* Feed study: standard "Conversion Factors" (mg/kg in feed : CF = mg/kg bw/day) of 7 and 20 have been used for mice and rats, respectively.

** Drinking water study: a standard "Conversion Factor" (mg/l in drinking water : CF = mg/kg bw/day) of 10 has been used for both mice and rats.

Table 1.5 *In vitro* genotoxicity tests with selected chlorophenols

Species or test system	End-point	Dose**	Purity[a] test subst.	Result*** without / with activation	Reference
2,4-DCP					
S. typh. TA98, 100, 1537	gene mut.	0-333 µg/plate$_t$	- 99%	[14] - / -*	Haworth et al. '83; NTP '89a
S. typh. TA1535	gene mut.	0-333 µg/plate$_t$	- 99%	[1] - / -*; ± [14]	Haworth et al. '83; NTP '89a
S. typh. TA98, 100, 1535, 1537, 1538	gene mut.	0-toxic conc.	-	[13] - / -	Simmon et al. '77
S. typh. TA98, 100, 1535, 1537, 1538, C3076, D3052, G46	gene mut.	10,000-fold range	-	- / -	Probst et al. '81
S. typh. TA98, 100, 1535, 1537	gene mut.	0-500 µg/plate$_t$	P, -	[14] - / -	Rasanen et al. '77
S. typh. TA100	gene mut.	0-1,000 µg/plate$_t$	-	[3] -	Rapson et al. '80
E. coli WP2, WP2uvrA	gene mut.	10,000-fold range	-	- / -	Probst et al. '81
Mouse lymphoma cells L5178Y	gene mut	0-60 µg/ml$_t$	-	+ / nt	NTP '89a
Chinese hamster cells V79	gene mut.	0-50 µg/ml$_t$	P,>99.5%	[18] -	Jansson & Jansson,'86
Chinese hamster ovary cells	chrom. ab.	0-75 µg/ml$_t$	-	- / -	NTP '89a
Chinese hamster ovary cells	SCEs	0-13 µg/ml$_{t/s}$	-	[8] ± / nt	NTP '89a
Chinese hamster ovary cells	SCEs	0-160 µg/ml$_{t/s}$	-	[8] nt / ±	NTP '89a
Rat hepatocytes	u-DNA-synth.	0-8 µg/ml$_t$	-	-	Probst et al. '81
2,6-DCP					
S. typh. TA98, 100, 1535, 1537	gene mut.	0-2,000 µg/plate$_t$	P,99%	[14] - / -*	Haworth et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-500 µg/plate$_t$	P, -	[14] - / -	Rasanen et al. '77
S. typh. TA100	gene mut.	0-1,000 µg/plate	-	[3] -	Rapson et al. '80
S. cere. D7, XV185-14C	gene mut.	-	-	- / nt	Nestmann & Lee '83
Chinese hamster cells V79	gene mut.	0/100 µg/ml	P,>99.9%	[16] -	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-150 µg/ml	P,>99.9%	[17] -	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-500 µg/ml$_t$	P,>99.5%	[18] -	Jansson & Jansson,'86
2,4,5-T3CP					
S. typh. TA98, 100, 1535, 1537	gene mut.	0-66 µg/plate$_{t/s}$	-	[14] - / -*	Haworth et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-500 µg/plate$_t$	P, -	[14] - / -	Rasanen et al. '77
S. typh. TA98, 100, 1535, 1537 1538	gene mut.	0-50 µg/plate$_{t/s}$	-	- / -	Nestmann et al. '80
S. typh. TA97, TA98	gene mut.	0-1,000 µg/plate$_t$	-	[14] - / +	Strobel & Grummt '87
S. typh. TA100	gene mut.	0-1,000 µg/plate$_t$	-	[14] + / -	Strobel & Grummt '87
S. typh. TA104	gene mut.	0-1,000 µg/plate$_t$	-	[14] - / -	Strobel & Grummt '87
S. cere. D7, XV185-14C	gene mut.	-	-	- / nt	Nestmann & Lee '83
Chinese hamster cells V79	gene mut.	0-50 µg/ml$_t$	P,>99.5%	[18] -	Jansson & Jansson,'86

(to be continued)

Table 1.5 In vitro genotoxicity tests with selected chlorophenols (continued)

Species or test system	End-point	Dose**		Purity@ test subst.	Result*** without / with activation		Reference
2,4,6-T3CP							
S. typh. TA98, 100, 1535, 1537	gene mut.	0-666	µg/plate$_{t/s}$	-	[14]	- / -*	Haworth et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-500	µg/plate$_t$	P, -	[14]	- / -	Rasanen et al. '77
S. typh. TA97	gene mut.	0-1,000	µg/plate$_t$	-	[14]	- / +	Strobel & Grummt '87
S. typh. TA98	gene mut.	0-1,000	µg/plate$_t$	-	[14]	- / +	Strobel & Grummt '87
S. typh. TA100	gene mut.	0-1,000	µg/plate$_t$	-	[14]	- / -	Strobel & Grummt '87
S. typh. TA104	gene mut.	0-1,000	µg/plate$_t$	-	[14]	- / +	Strobel & Grummt '87
S. typh. TA100	gene mut.	0-1,000	µg/plate	-	[3]	-	Rapson et al. '80
S. cere. MP-1	gene mut.	400	µg/ml	P, 99%	[11]	±	Fahrig et al., 78
House lymphoma cells L5178Y	gene mut.	0-200	µg/ml	-		+ / nt	McGregor et al. '88
Chinese hamster cells V79	gene mut.	0-60	µg/mg$_t$	P,>99.9	[15]	+	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0/30	µg/ml	P,>99.9%	[16]	-	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-100	µg/ml$_t$	P,>99.5%	[18]	-	Jansson & Jansson,'86
CHO cells	chrom. ab.	0-500	µg/ml$_t$	-		- / -	Galloway et al. '87
CHO cells	SCEs	0-50	µg/ml	-		- / nt	Galloway et al. '87
CHO cells	SCEs	0-500	µg/ml	-		nt / -	Galloway et al. '87
2,3,4,6-T4CP							
S. typh. TA98, 100, 1535, 1537	gene mut.	0-500	µg/plate$_t$	P, -	[14]	- / -	Rasanen et al. '77
S. typh. TA97, 98, 100, 1535	gene mut.	0-100	µg/plate$_t$	T, 86%		- / -	Zeiger et al. '88
Chinese hamster cells V79	gene mut.	0-20	µg/mg	P,>99.9	[15]	+	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0/10	µg/ml	P,>99.9	[16]	-	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-20	µg/ml	P,>99.9	[17]	-	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-100	µg/ml$_t$	P,>99.5%	[18]	-	Jansson & Jansson,'86
PCP							
S. typh. TA98, 100, 1535, 1537	gene mut.	0-30	µg/plate$_t$	T, 92%	[14]	- / -*	Haworth et al. '83; NTP '89b
S. typh. TA98, 100, 1535, 1537, 1538	gene mut.	0-5,000	µg/plate	-	[2]	-	Moriya et al. '83
S. typh. 8 his$^-$ strains	gene mut.	-		T, ≥90%	[3]	-	Anderson et al. '72
S. typh. TA98, 100, 1535, 1537, 1538	gene mut.	0-toxic conc.		-	[13]	- / -	Simmon et al. '77
S. typh. TA1535, 1536, 1537, 1538	gene mut.	-		-	[3]	-	Shirasu, 1975
S. typh. TA98,	gene mut.	0-27	µg/plate$_t$	-		- / ±	Nishimura et al. '82
S. typh. TA100	gene mut.	0-27	µg/plate$_t$	-		- / -	Nishimura et al. '82
S. typh. G 46	gene mut.	-		-	[3]	-	Buselmaier et al. '72
E. coli WP2 hcr	gene mut.	0-5,000	µg/plate	-	[2]	-	Moriya et al. '83
E. coli B/r WP2 hcr$^+$, hcr$^-$	gene mut.	-		-		-	Shirasu, 1975
E. coli	gene mut.	-		-	[2,5]	-	Fahrig '74-
E. coli Gal R^S	gene mut.	-		-	[2,6]	-	Fahrig '74-
S. marc. a 21, a 742	gene mut.	-		-	[2,6]	-	Fahrig '74-
S. marc. a 21	gene mut.	-		-	[3]	-	Buselmaier et al. '72
S. cere. MP-1	gene mut.	400	µg/ml$_t$	P, 99%	[12]	+	Fahrig et al., '78
S. cere. MP-1	gene conv.	400	µg.ml$_t$	P, 99%	[12]	+	Fahrig et al., '78
S. cere.	gene conv.	50	µg/ml$_t$	-	[2,4]	+	Fahrig '74
B. subt. Rec$^+$, Rec$^-$	"DNA damage"	-		-		-	Shirasu, '75

(to be continued)

Table 1.5 In vitro genotoxicity tests with selected chlorophenols (continued)

Species or test system	End-point	Dose**		Purity@ test subst.	Result*** without / with activation	Reference
PCP (continued)						
Chinese hamster cells V79	gene mut.	0/15	µg/ml	P,>99.9 [16]	-	Hattula & Knuutinen'85
Chinese hamster cells V79	gene mut.	0-50	µg/ml t	P,>99.5% [18]	-	Jansson & Jansson,'86
Chinese hamster ovary cells	chrom. ab.	0-100	µg/ml t/s	T, 92% [10]	- / ±	Galloway et al. '87; NTP '89b
Chinese hamster ovary cells	SCEs	0-30	µg/ml t/s	T, 92% [9]	- / -	Galloway et al. '87;
Human lymphocytes	chrom. ab.	-		- [2,7]	±	Fahrig '74~ NTP '89b
NaPCP						
Human lymphocytes	SCEs	0-90	µg/ml t	T, 85% [3]	-	Ziemsen et al. '87
Human lymphocytes	chrom. ab.	0-90	µg/ml t	T, 85% [3]	-	Ziemsem et al. '87

Result:
positive response: +
negative response: -
equivocal response: ± (weakly positive and/or not dose-related and/or not reproducible response)

Species:
B. subt. = Bacillus subtilis
E. coli = Escherichia coli
S. cere. = Saccharomyces cerevisiae
S. marc. = Serratia marcescens
S. typh. = Salmonella typhimurium

End-point:
gene mut. = gene mutation
gene conv. = gene conversion
chrom. ab. = chromosomal aberrations
SCEs = sister chromatid exchanges
u-DNA-synth. = unscheduled DNA-synthesis

* In these NTP studies both rat and hamster liver S9 mix were used separately as metabolic activation systems; the response indicated is the combined result of these tests.

** The highest dose tested is limited by toxicity (t) or solubility (s).

*** In a number of studies several species and/or strains have been tested separately; in these cases the response indicated is the combined result of all tests (either without or with metabolic activation).

@ P: "purified"; T: "technical-grade".

For further footnotes, see next page.

[1] Negative response in two tests with rat S9; weakly positive/equivocal response in two tests with hamster S9-mix.

[2] In this study a great number of pesticides has been tested; according to the section on "materials and methods", S9-mix has been used when required, but the results of tests without and with metabolic activation have not been reported separately.

[3] Presence of metabolic activation not stated.

[4] Induction of mitotic gene conversion at the "ade2" and "trp5" loci, after a 6-hr treatment time in a liquid holding test. At the test concentration used (0.19 mM in 1% DMSO) survival was 30%. No other data on "materials and methods" and "results" are reported.

[5] Liquid holding test, detecting a forward mutation to streptomycin-resistance in E. coli (not available, based on personal communication to Fahrig).

[6] Spot tests, detecting a reverse mutation to prototrophy in S. marcescens or a forward mutation to galactose prototrophy in E. coli (not available, based on personal communication to Fahrig).

[7] Not available, based on personal communication to Fahrig.

[8] In this study the test concentrations used in the tests with and without metabolic activation were different (so, "nt" in this study stands for "not tested at this concentration range"). Based on their definitions, "NTP" considered the response in these test to be "positive", but this conclusion is not supported by "RIVM"-experts on genotoxicity.

[9] Based on their definitions, "NTP" considered the response in the test without metabolic activation to be "weakly positive", but this conclusion is not supported by "RIVM"-experts on genotoxicity.

[10] Based on their definitions, "NTP" considered the response in the test with metabolic activation to be "positive", but this conclusion is not supported by "RIVM"-experts on genotoxicity.

[11] Fahrig et al. considered the response (a two-fold increase; significant at $p < 0.02$) to be "very weak".

[12] A three-fold and two-fold increase (both significant at $p < 0.001$) were observed with regard to forward gene mutation and mitotic gene conversion (intragenic recombination), respectively.

[13] Reagents of the highest available purity were perchased from commercial suppliers.

[14] Test compound dissolved in dimethyl sulphoxide (DMSO).

[15] Direct assay: test compound added to a monoculture of V79 cells. Solvent: acetone. Exposure time: 48-hr. The historical background mutant frequency has not been reported. The mutant frequency at exposure was up to 35×10^{-6} and 53×10^{-6} for 2,3,4,6-T4CP and 2,4,6-T3CP, respectively; that in control groups of these tests was 0×10^{-6} (In other control groups in this test system, the mutant frequency was up to 34×10^{-6}).

[16] Hepatocyte-mediated assay: test compound added to a culture of V79 cells and rat hepatocytes. Solvent: acetone. Exposure time: 48-hr.

[17] Fibroblast-mediated assay: test compound added to a culture of V79 cells and rat fibroblasts. Solvent: acetone. Exposure time: 48-hr.

[18] Direct assay: test compound added to a monoculture of V79 cells. Solvent: acetone. Exposure time: 24-hr.

Table 1.6 In vivo genotoxicity tests with chlorophenols - animal studies

Species and test system	Exposure	Exp time	Result	Reference
2-MCP				
Mouse (m,f; adult) Sperm morphology, SCEs and other effects [6]	0-35-69-175 mg/kg bw/day by gavage, in corn oil	2-w	Negative	Borzelleca, '85c [1a]
2,3-DCP				
Mouse (m) Bone marrow SCEs	Single toxic (sublethal) dose, i.p. injection	26-hr	Negative	Kessler et al.
2,4-DCP				
Mouse (m) Bone marrow SCEs	Single toxic (sublethal) dose, i.p. injection	26-hr	Negative	Kessler et al.
Mouse (m,f; adult) Sperm morphology, SCEs and other effects [6]	0-64-128-638 mg/kg bw/day by gavage, in corn oil	2-w	Negative	Borzelleca, '85c [1b]
2,5-DCP				
Mouse (m) Bone marrow SCEs	Single toxic (sublethal) dose, 80-240 mg/kg bw; i.p. injection	26-hr	Equivocal	Kessler et al., [5]
2,6-DCP				
Mouse (m) Bone marrow SCEs	Single toxic (sublethal) dose, i.p. injection	26-hr	Negative	Kessler et al.
2,4,6-TCP				
Mouse (f, age 10 w) Spot test	50 or 100 mg/kg/bw, i.p. injection on day 10 of gestation (40 animals/group)	foetal period	Negative	Fahrig et al., '78 [4]

(to be continued)

Table 1.6 In vivo genotoxicity tests with chlorophenois - animal studies (continued)

Species and test system	Exposure	Exp time	Result	Reference
PCP				
Mouse (age 10-12 w) Host-mediated assay (gene mutation in S. marc strain a 21 and S. Typh, strain G46	75 mg/kg bw, s.c.	3-hr [1]	Negative in both strains	Buselmaier et al.,'72
Mouse (m) Bone marrow SCEs	Single toxic (sublethal) dose, i.p. injection	26-hr	Negative	Kessler et al.
Mouse (f, age 10 w) Spot test	50 or 100 mg/kg/bw, i.p. injection on day 10 of gestation (40 animals/group)	foetal period	Negative	Fahrig et al., '78 [3]
Mouse (m, age 7-10w) Sperm morphology assay	6-400 mg/kg bw/day, i.p. injections on each of 5 consecutive days	5-w	Negative, both with reagent- and technical-grade PCP	Osterloh et al.,'83 [2]
Drosophila melanogaster Sex-linked lethal test	2,000 mg/l in feeding solution	3-d	Negative	Vogel & Chandler,'74
Drosophila sp.	sublethal concentration of 400 ppm in corn meal-agar substance	larval period	Negative (no effect on nondisjunction and loss of sex chromosomes	Ramel & Magnusson,'79

S. marc. = Serratia marcescens; S typh. = Salmonella typhimurium

[1a] The highest concentration tested was lethal (see table 1.2).
[1b] The highest concentration tested did not result in toxicity (see table 1.2).
[2] Highest sublethal dose: 50 mg/kg bw/day. Lowest lethal dose: 100 mg/kg bw/day.
[3] In surviving offspring the total incidences of spots of genetic relevance (indicating an alteration of the wild type allele of one of the 4 "color genes" under study or its loss) were 1/169 and 1/147 in two tests with a dose level of 50 mg/kg bw, and 2/157 in one test at a dose level of 100 mg/kg bw. The incidence in controls was 1/967.
[4] In surviving offspring the total incidences of spots of genetic relevance (see [3]) were 1/181, 1/159 and 1/175 at a dose level of 50, 50 and 100 mg.kg^{-1} bw; the incidence in controls was 1/967.
[5] The increase in SCEs was dose-related. However, only at the highest dose level tested there was a two-fold increase over baseline readings (7.3 SCEs/cell versus 3.3 SCEs/cell); this concentration was cytotoxic.
[6] Sperm morphology, testicular DNA synthesis, sister chromatid exchanges in testis and bone marrow, and mitotic index in bone marrow.

2 ECOTOXICITY - I: AQUATIC ORGANISMS

2.1 ACCUMULATION

Studies on the accumulation in freshwater and marine organisms has been reviewed recently for PCP (WHO, 1987) and for chlorophenols other than PCP (WHO, 1989). The main results, based on both laboratory and field studies, are reported below together with some (additional) information based on primary literature sources.

PCP

For algae and invertebrates, bioconcentration factors (BCFs) up to about 1,000 have been reported generally *[The BCF is the concentration in organisms (weight.kg^{-1} fresh weight) divided by the concentration in water (weight.l^{-1})]*. Considerably higher BCFs have been reported for the marine polychaete worm *Lanice conchilega*. In a field study in which these worms were collected from a location in the Wadden sea, whole-body BCFs of 2,600 to 8,500 (based on wet weights of organisms) were calculated on the basis of an average ambient concentration of 0.04×10^{-3} $\mu g.l^{-1}$; whole-body BCFs for another bottom living animal, the actinian *Sagartia troglodytes*, were much lower (70 to 180) although both species were collected from the same sampling locations (Ernst and Weber, 1978). The high potential of *L. conchilega* to concentrate PCP was confirmed in a static laboratory test in which whole-body BCFs were calculated on the basis of the steady-state concentration in the water: exposure to an initial concentration of 2-5 $\mu g.l^{-1}$ resulted in a BCF of 3,800 for this species while a 10-times lower BCF was found for the mussel *Mytilus edulis* (Ernst, 1979).

For freshwater fish, whole-body BCFs in the range of 100 to 1,000 have been calculated, based on short-term (up to 5 days) studies in which the fish were exposed to concentrations of about 50 to 200 $\mu g.l^{-1}$. Similar studies with regard to exposure time and concentration have resulted in BCFs in the range of 10 to 100 for marine fish (WHO, 1987). In a long-term study with freshwater fish, rainbow trout *Salmo gairdneri* was exposed to concentrations of 0.01 (control), 0.035 or 0.66 μg NaPCP.l^{-1} in continuous flow systems; the concentrations were chosen on the basis of those measured in natural environments. Exposure to 0.035 μg NaPCP.l^{-1} resulted in whole-body BCFs of 750 and 200, after 6 and 16 weeks, respectively. At 0.66 μg NaPCP.l^{-1}, the highest whole-body BCF (260) was found after 13 weeks of exposure; after 16 weeks a slightly lower whole-body BCF (240) was found (Niimi and McFadden, 1982). Another long-term study on the accumulation of

PCP was conducted with marine fish. In surviving adult sheepshead minnows, *Cyprinodon variegatus*, exposed in a continuous-flow system from the egg stage through 5 months of age to measured concentrations ranging from 18 to 195 μg PCP.l^{-1}, whole-body BCFs ranged from 5 to 27. In surviving 28-d old F1-juveniles, whole-body BCFs ranged from 16 to 48 (Parrish et al., 1978).

Abiotic factors which strongly influences the accumulation of PCP, are the pH and, at high pH-values, the ionic strength, the two factors governing the partition coefficient (WHO, 1987). For example, a 1-hr exposure of goldfish *Carassius auratus* to 0.1 mg PCP.l^{-1} at pH-values of 5.5, 6, 7, 8, 9 and 10 resulted in BCFs of 131, 120, 56, 24, 12 and 2, respectively. Correspondingly, toxicity of PCP decreased with increasing pH (Kobayashi and Kishino, 1980).

Chlorophenols other than PCP

Little data appear to be available on the accumulation of these compounds. A study in which a freshwater "microcosm" was exposed for 5 weeks to 0.5 μg 2,4,6-T3CP in a continuous-flow system resulted in BCFs of 1,000-4,500 for macrophytes, 3,000 for invertebrates and 1,000-12,000 for fish. Another long-term study using a freshwater microcosm (exposed for 4 weeks to 0.5 or 5 mg 2,4,5-T3CP.l^{-1}) resulted in BCFs up to about 2,000 for fish. Short-term (up to 3 days) laboratory studies with fish resulted in BCFs up to 500 for miscellaneous chlorophenols (WHO, 1989). In some studies a trend of higher BCFs with increasing chlorination has been observed, but it is noted that in these studies the exposure concentration decreased with increasing chlorination. For example, exposure of goldfish *C. auratus* for 12 hours to a number of chlorophenols at lethal concentrations ranging from 60 mg.l^{-1} for 2-MCP to 0.2 mg.l^{-1} for PCP, resulted in BCFs (based on the concentrations measured in dead fish) of 6-10 for MCP, 34 for 2,4-DCP, 20-60 for T3CP, 93 for 2,3,4,6-T4CP and 475 for PCP. The exposure concentrations for each compound were chosen closely to the 24-hr LC50-values (Kobayashi et al., 1979).

Considerably higher BCFs were calculated for the marine polychaete worm *L. conchilega:* 11,000 to 25,000 for T3CP and T4CP, at very low ambient concentrations (low picogram.l^{-1} range); these BCFs are based on the concentration potential compared to PCP. The concentration potential of T4CP and T3CP was 4-6 and 7-8 times higher, respectively, than that of PCP,

at ambient concentrations that were 1-15% of that of PCP (Ernst and Weber, 1979).

2.2 TOXICITY

Introduction

In this section a distinction has been made between freshwater organisms and marine organisms (both seawater and estuarine organisms), and between "short-term" and "long-term" exposure. Short-term exposure covers data on experiments with exposure times up to 96 hours; the most relevant endpoint of "acute toxicity" studied in these experiments is lethality. Long-term exposure preferably covers data on experiments in which organisms are exposed during a significant part of their lifetimes or, at least, during a sensitive life stage. The most relevant endpoints of "chronic toxicity" studied in these latter experiments are effects on growth and reproduction, at sublethal concentrations. Some organisms (bacteria, algae) do have very short lifetimes; therefore the section on long-term exposure also includes ≤ 96-hr experiments with this kind of organisms, in case the exposure time covers one or more generations.

Most "single species" toxicity tests are summarized in the tables 2.1 through 2.5. With exception of two tests in table 2.5, all tests were evaluated on the basis of the primary literature source and are conducted according to current guidelines for aquatic toxicity testing. A number of the tests summarized in table 2.6 do not meet current guidelines or could not be evaluated because of limited data reported, but these tests have been summarized in this table (together with tests from the afore-mentioned tables), to show the relative toxicity of individual chlorophenols under identical test conditions.

For PCP, a large number of long-term toxicity tests with freshwater organisms are available, each resulting in a no-observed-effect-concentration (NOEC) with regard to relevant sublethal parameters. Because long-term NOEC-values are used preferably to establish a maximum acceptable concentration ("limit value") in surface water, the vast amount of short-term toxicity tests with PCP was not evaluated. A brief review of toxicity values (LC50- and EC50-values) for PCP is given in the text.

In the draft of the "Integrated Criteria Document Chlorophenols" (January 1990) it has been concluded that current and expected exposure levels of chlorophenols in surface waters in the Netherlands are much lower than the

pentachlorophenol copper salt (added as 2% or 42% liquid) or "Dowicide EC-7" (commercial PCP-formulation, purity 88%) resulted in similar 48-hr and 96-hr L(E)C50-values, namely 26 to 920 $\mu g.l^{-1}$. All values mentioned are based on measured concentrations (Mayer, Jr. and Ellersieck, 1986).
In the third study the sensitivity to "Dowicide EC-7" (94% PCP) of 11 native species (molluscs, crustaceans, insects and fish) was studied in flow-through tests. Most tests were seasonal toxicity tests in river water, conducted under ambient water temperature and quality; the ranges of water characteristics (over all tests) were as follows: temperature 3 to 25 °C; pH 7.4 to 8.4; hardness 112 to 196 $mg.l^{-1}$). Additional tests were conducted in lake water under controlled conditions (temperature 25 °C; ambient pH 7.3; adjusted pH 7.7 to 8.4). In all, 51 tests were conducted, resulting in 48/96-hr LC50-values ranging from 85 $\mu g.l^{-1}$ for the fish *Catastomus commersoni* to >7,770 $\mu g.l^{-1}$ for the crustacean *Asellus racovitzai*. Most values were below 500 $\mu g.l^{-1}$; seasonality (influence of both temperature and life stage) influenced the sensitivity of some species. Over all seasons, the three fish species tested were most sensitive, followed by the three cladoceran species (Hedtke et al., 1986).
The susceptability of different early life stages of rainbow trout *Salmo gairdneri* to PCP (99%) was investigated in renewal tests in artificial (reconstituted) test water (pH 7.2; hardness 50 $mg.l^{-1}$). The 96-hr LC50-values were ranging from 480 to 3,000 $\mu g.l^{-1}$ for different egg stages. Fry were considerable more sensitive: 96-hr LC50-values for sac fry and early fry were 32 and 18 $\mu g.l^{-1}$, respectively (Van Leeuwen et al., 1985).

In several studies it has been observed that the acute toxicity of PCP and that of chlorophenols other than PCP is depending on the pH-value of the test water. For example, in tests with the goldfish *carassius auratus*, lethal toxicity of PCP was found to decrease with increasing pH-value of the test water: at pH-values of 5.5, 6, 7, 8, 9 and 10, 24-hr LC50-values were 0.05, 0.06, 0.08, 0.25, 2.2 and 16 $mg.l^{-1}$, respectively. Correspondingly, the accumulation of PCP was found to decrease with increasing pH-value (Kobayashi and Kishino, 1980). Similarly, Spehar et al. (1985) reported differences of a factor of 4-10 between 96-hr LC50-values at pH-values of 6.5 and 8.5, for different organisms exposed to PCP. Könemann (1979) and Saarikoski and Viluksela (1981, 1982) studied the influence of pH on the toxicity of miscellaneous chlorophenols to fish (see also table 2.6); these studies show that the toxicity of chlorophenols decreases with increasing pH-value. Generally, the pH-effect decreases with

decreasing chlorination, consistent with the increase in pKa-value (acid dissociation constant) with decreasing chlorination.

Chlorophenols other than PCP - table 2.1

Short-term toxicity tests (freshwater organisms) resulting in L(E)C50-values are summarized in table 2.1.
For the influence of the pH-value on the acute toxicity of chlorophenols, the reader is referred to the afore-mentioned data (see PCP).

Quantitative structure-activity relationships (QSARs)

The relationship between physico-chemical properties of chlorophenols and their toxicity, especially lethal toxicity at short-term exposure, has been investigated in a number of studies, for example by Könemann (1979), Liu et al. (1982), Kaiser et al. (1984), Devillers and Chambon (1986), Banerjee (1987), Leblanc et al. (1988), Shigeoka et al. (1988a,b) and Zomer et al. (1990). These studies, in which toxicity data on different organisms - bacteria, algae, crustaceans or fish- were used, show that the toxicity generally increases with increasing chlorination. QSAR-equations based on regression analyses show that the toxicity (expressed as molar concentration) is related primarily to the lipophilicity (expressed as the logarithm of the n-octanol water partition coefficient, $P_{o/w}$ or P_{oct}). The strong, positive correlation between toxicity and this variable indicates that the toxicity of chlorophenols is primarily caused by a non-specific mode of action, "physical effect", with little dependence on chemical structure (consistent with QSAR-studies using a variety of other nonpolar chemicals). Könemann (1979) exposed fish to a mixture of phenol and 10 chlorophenols and showed that the toxicity of the mixture could be calculated on the basis of the concentrations and LC50-values of each compound present in the mixture. Therefore, phenol and the chlorophenols showed the additive action to be expected on the basis of a non-specific mode of action. The QSAR-studies also show that the toxicity of chlorophenols is not only dependent on the number of chlorine atoms, but also on the position thereof (see also table 2.1, 2.2 and, especially, table 2.6). For example, comparative studies show that within the group of dichlorophenols the toxicity of the *para*- and *meta*- substituted compounds is consistently higher than that of the *otho*- substituted compounds. Accordingly, multiple linear correlations which include (beside log $P_{o/w}$)

other variables such as the acid dissociation constant (pK_a) and/or Hammett's constant for ortho substitution ($\sum\delta$) may result in better correlations.

In some studies there is a deviation of the general trend of increasing toxicity with increasing chlorination. For example, in a study with two different species of algae, the 96-hr EC50 (growth inhibition) for one of these species increased from MCP (170-30 $mg.l^{-1}$) to DCP (10 $mg.l^{-1}$), but a further increase in the number of chlorine atoms up to PCP did not result in a further increase in toxicity (Shigeoka et al., 1988a; table 2.2). A second example is also reported by Shigeoka et al. (1988b): 14-d life cycle studies with waterflea *D. magna* resulted in similar NOEC-values for reproduction, independent of chlorination. However, there was a clear trend of increasing toxicity with increasing chlorination for immobilization, both after 24 hours and 14 days (table 2.6). This indicates that reproduction is influenced by a different mode of action than immobilization.

Long-term exposure ("single species" tests)

L(E)C50-values (table 2.2)

Long-term toxicity tests (freshwater organisms) resulting in L(E)C50-values are summarized in table 2.2. In this table both data on PCP and on other chlorophenols are listed. The exposure time ranged from 4 days (algae) to 21 days (crustaceans).

Additional data on L(E)C50-values

In an ISO-test program, the toxicity of 3,5-DCP to 2 species of unicellular green algae, *Selenastrum capricornutum and Scenedesmus quadricauda,* was studied by 4 different laboratories which were allowed to use their common test method. The resulting EC50-values (parameter: growth inhibition) for *S. capricornutum* and *S.quadricauda* were ranging from 1,200 to 7,500 $\mu g.l^{-1}$ and from 770 to >10,500 $\mu g.l^{-1}$, respectively. In the tests involved, exposure times ranged from 4 to 18 days (Hanstveit, 1980).

NOEC-values (table 2.3 and table 2.4)

PCP (table 2.3)

Long-term toxicity tests (freshwater organisms) resulting in NOEC-values are summarized in table 2.3. In the text below some studies listed in this table are discussed.

In a comparative study, the toxicity of three different PCP-formulations to fry of fathead minnow *Pimephales promelas* was investigated using partial life-cycle tests. The resulting NOEC-values were 6, 36 and 139 μg PCP.l^{-1} for a composite of commercial available "technical-grade" PCPs, "purified" PCP (99% PCP) and "Dowicide EC-7" (91% PCP), respectively. The relatively high toxicity of the mixture of "technical grade" PCPs and the specific effects (degeneration of fins and opercles, malformations of the anterior regions of the skull) found at exposure to this preparation, are probable associated with the presence of relatively high concentrations of highly toxic contaminants such as chlorinated phenoxyphenols, and polychlorinated dibenzo-p-dioxins, PCDD, and dibenzofurans, PCDF (Cleveland et al., 1982). A follow-up study (Hamilton et al., 1986) using the same fish species and identical test methods, resulted in an NOEC of 66 μg PCP.l^{-1} and a (marginal) effect-concentration of 130 $\mu g.l^{-1}$, using an "ultrapurified" PCP-formulation (purity > 99%) preparation containing less chlorinated phenoxyphenols than the PCP-formulation used in the study by Cleveland et al. (1982). In addition, Hamilton et al. (1986) studied the toxicity of a mixture of chlorinated phenoxyphenols, isolated from the mixture of "technical-grade" PCPs tested by Cleveland et al. (1982); nominal exposure concentrations of the phenoxyphenols were based on those in the study by Cleveland et al. (1982). The results of the above-mentioned studies indicate that chlorinated phenoxyphenols may contribute significantly to the effects of "technical-grade" PCP.

In an embryo-larval test in which trout *Salmo gairdneri* was exposed to NaPCP prepared from "purified" PCP (> 99%), yolk sac edema and cranial malformations were rare, while these effects are commonly observed in similar tests with "technical-grade" PCP (Dominguez and Chapman, 1984).

In a number of tests, different stages of steelhead trout *Salmo gairdneri* were exposed to Santobrite^{R} containing a minimum NaPCP content of 90%. All experiments were conducted in filtered stream water with pH $\pm$ 7.8. The following results are expressed as μg PCP.l^{-1} , calculated on the basis of nominal concentrations of the test compound and assuming 90% purity.

Semi-static tests with embryos, exposed from fertilization to shortly after hatching, resulted in 100% mortality at all concentrations tested ($\geq$ 33 μg PCP.l^{-1}). In these tests, concentrations of 33 and 66 μg PCP.l^{-1} resulted in mortality after hatching, while higher concentrations resulted in embryo mortality. In flow-through tests in which alevins were exposed for 7 weeks to 33 μg PCP.l^{-1}, percentage mortality increased with decreasing oxygen levels; the dissolved oxygen levels, ranging from 3 to 10 mg O_2.l^{-1}, were of themselves non-lethal. The combination of 33 μg PCP.l^{-1} and 10 mg O_2.l^{-1} resulted in 25% mortality. In additional flow-through tests, embryos were exposed from fertilization to the time of complete yolk utilization; embryos and alevins were exposed to 8, 17 and 33 μg PCP.l^{-1}, each in water with dissolved oxygen levels of 3, 5, or 10 mg O_2.l^{-1}. All combinations of PCP and oxygen resulted in increased mortality (when compared to the oxygen control), with exception of the combination of 8 μg PCP.l^{-1} and 10 mg O_2.l^{-1}. The highest concentration tested resulted in 100% mortality, regardless of the oxygen level. The concentration of 17 μg PCP.l^{-1} reduced maximum dry weights of surviving alevins, especially at low oxygen level. The concentration of 8 μg PCP.l^{-1} slightly reduced weight at low oxygen level, but was without effect at the oxygen level of 10 mg O_2.l^{-1} (Chapman and Shumway, 1978).

In the experiment by Hodson and Blunt (1981) the interaction between NaPCP and temperature was studied in tests with trout *S. gairdneri*. Exposure temperatures for eggs, alevins and fry were 5 or 10 °C, 5 or 15 °C, and 12 or 20 °C, respectively; mean measured concentrations were 0.25 (controls), 13, 24 and 80 μg.l^{-1}. The experiment was started with either fertilized eggs or post-hatch alevins. Temperature significantly ($p \leq 0.01$) enhanced the effects of NaPCP (weight of alevins at hatch, weight at swim-up, yolk sac resorption efficiency, grow rate during feeding stage). However, biomass of eggs exposed at the lowest temperature was most affected by the highest concentration, and the alevins originating from these eggs failed to develop to the swim-up stage. The relatively high sensitivity at low temperature may be the result of the prolonged egg development time.

Chlorophenols other than PCP - Table 2.4

Long-term toxicity tests (freshwater organisms) resulting in NOEC-values are summarized in table 2.4.

Additional data ("single species" tests)

PCP

-In a comparative study, the effects of "Dowicide EC-7" (94% PCP) on growth and/or reproduction of 7 species (molluscs, crustaceans, fish, plants) were studied in river or lake water. Reproduction of the cladoceran *Ceriodaphnia reticulata* and the snail *Physa gyrina* were significantly affected at the lowest test concentration: 4.1 and 26 $\mu g.l^{-1}$, respectively (actual concentrations). For the remaining 5 species, NOEC-values ranged from 75 $\mu g.l^{-1}$ for the cladoceran *C. affinis/dubia* to >1,440 $\mu g.l^{-1}$ for the plant *Lemna minor* (Hedtke et al., 1986). *These NOEC-values are not listed in table 2.3, and have not been used in the risk assessment, because the publication was received shortly before the dead-line of this report.*

-A concentration of 1 μg PCP.l^{-1} has been reported to reduce the fertility of *D. magna* (Kolosova and Stroganov, 1973). Because the article is in Russian, this information can not be evaluated.

-In static tests, sexually mature snails of two strains of *Australorbis glabratus* were exposed to concentrations of 50 and 100 μg NaPCP.l^{-1} for 7 days. At 50 μg NaPCP.l^{-1}, fecundity of one strain was adversely affected; the viability of the eggs of both strains was greatly reduced. Exposure to 100 μg NaPCP.l^{-1} resulted in increased snail mortality, and greatly reduced both fecundity and egg viability of both strains. Transfer of the snails to untreated water after exposure resulted in a partial recovery (Olivier and Haskins, 1960).

-Increased mortality, reduction of growth, and retardation or complete inhibition of sexual maturity was found at exposure of common yuppies *Lebistes reticulatus* to 500 μg "technical-grade" NaPCP.l^{-1} for 90 days in a renewal test. The fish were < 2-d old at start; pH and total hardness of the test water were 8.5 and 165 $mg.l^{-1}$, respectively. Lower concentrations were not tested (Crandall and Goodnight, 1962).

Chlorophenols other than PCP

Tests with goldfish *C. auratus* exposed for 8 days (4-d embryonal exposure and 4-d larval exposure) to 2,4-DCP in a continuous-flow system resulted in LC50-values of 390 and 260 mg 2,4-DCP.l^{-1}, at a water hardness of 50 and 200 $mg.l^{-1}$ $CaCO_3$, respectively. Identical tests with channel catfish *I. puntatus* resulted in LC50-values of 1,350 and 1,070 $mg.l^{-1}$, respectively. The pH of the test water was 7.8. Tests with rainbow

trout *S. gairdneri* exposed for 28 days (24-d embryonal exposure and 4-d larval exposure) to 2,4-DCP in a continuous-flow system resulted in LC50-values of 80 and 70 mg 2,4-DCP.l^{-1}, at a water hardness of 50 and 200 mg.l^{-1}, respectively (cited in Krijgheld and van der Gen; primary source not available).

Field and ecosystem studies ("multiple species" tests)

PCP

The effect of a concentration of 20 μg NaPCP.l^{-1} on fish *Pseudorasbora parva* (initial length and weight: 4 cm and 1 g, respectively) and on the number and weight of several groups of invertebrates present in the well water used, was studied in artificial streams. Preceeding exposure, water was flown through the system without fish, to provided sufficient food organisms for the fish. After 6 weeks of exposure to NaPCP, mortality and growth of the fish were not affected (the fish received no additional food during the experiment). In this test, the number and weight of benthic animals in control and test streams were similar. In a second experiment, the effects on mortality and growth of juvenile sweet fish *Plecoglossus altivelis* and on hatching of eggs of this fish were studied in these artificial streams, in three consecutive experiments. In these experiments, the fish received additional food daily. Exposure concentrations and exposure times were 2, 20 and 34 μg NaPCP.l^{-1}, and 14, 20 and 16 weeks, respectively. At 34 μg NaPCP.l^{-1}, mortality was not affected; the other parameters appeared to be adversely affected, especially hatching. The lowest two concentrations were without apparent effects (statistical data are not reported). Treatment-related histological effects were not observed. In a third experiment, mortality and growth of fry of common carp *Cyprinus carpio* exposed for 10 weeks in outdoor ponds (continuous-flow exposure) were not affected at 20 μg NaPCP.l^{-1}. Other concentrations were not tested (Matida et al., 1970).

The results of four field studies on the effects of PCP on (experimental) ecosystems have been evaluated by Okkerman et al. (1990). In three of these studies, effects were observed at the test concentrations used: 40 μg.l^{-1} (purity 94%), 100 μg.l^{-1} (purity not reported) and 500 μg.l^{-1} (purity not reported), respectively. In the fourth study, some plant species were strongly affected at a repeated exposure to 67 μg.l^{-1} (purity > 99%), while

invertebrates appeared not to be affected; a concentration of 20 $\mu g.l^{-1}$ was without effect. In this study PCP was applied every 3 or 4 days.

2.2.2 Marine organisms ("single species" tests)

Short-term and long-term "single species" toxicity tests with marine organisms are summarized in table 2.5. The data are very limited, especially for chlorophenols other than PCP.

Additional data

Conklin and Rao (1978) reviewed a number of 96-hr LC50-values for PCP, derived from tests with crustaceans. With regard to marine crustaceans, the lowest values (84 to 363 μg PCP.l^{-1}) were reported for larvae.

2.2.3 Relative toxicity chlorophenols (freshwater and marine organisms)

In a large number of studies, the toxicity of several individual chlorophenols has been studied under identical test conditions. These studies are summarized in table 2.6 (see also "short-term exposure", QSARs).

Summary and conclusions "aquatic organisms"

Accumulation

For PCP, bioconcentration factors (BCFs) up to about 1,000 are usually reported for both freshwater and marine organisms, including algae, invertebrates and vertebrates (fish). For chlorophenols other than PCP, both similar and higher BCFs have been reported. On the basis of the limited data available it is concluded that PCP is accumulated (concentrated) to a limited extend by aquatic organisms and that PCP appears to have a low potential for biomagnification in the aquatic environment. *[Biomagnification is the occurence of a substance at successive higher concentrations with increasing trophic levels in food chains]* However, data on aquatic vertebrates other than fish are lacking. A number of chlorophenols other than PCP appear to have a higher potential for bioaccumulation, but the data for these compounds are very limited.

Toxicity to freshwater organisms

The majority of the data refer to PCP; this compound has been studied in a large number of short-term and long-term "single species" toxicity tests. Test organisms included algae and a variety of invertebrates and vertebrates (fish). Data on chlorophenols other than PCP are limited, especially with regard to long-term tests in which sublethal parameters were studied.

The lowest 48/96-hr L(E)C50-values from "single species" tests conducted according to current guidelines are 2,500 $\mu g.l^{-1}$ for MCP, 1,400 $\mu g.l^{-1}$ for DCP, 900 $\mu g.l^{-1}$ for T3CP, 205 $\mu g.l^{-1}$ for T4CP and 18 $\mu g.l^{-1}$ for PCP. Generally, the toxicity increases with increasing chlorination, but the toxicity is also dependent on other physico-chemical properties such as the position of the chlorine atoms, the *para-* and *meta-* substituted compounds being more toxic than the *ortho* -substituted compounds. The toxicity decreases with increasing pH-value of the water, especially the toxicity of the higher chlorinated compounds which have the lowest pK_a-values.

Long-term "single species" tests with PCP (n = 26) have resulted in a wide range of NOEC-values, 3 to 3,200 $\mu g.l^{-1}$, with regard to sublethal parameters such as growth and reproduction. About half of these NOEC-values was below 50 $\mu g.l^{-1}$. The lowest NOEC-values for chlorophenols other than PCP (based on 1 to 3 tests) are 500 $\mu g.l^{-1}$ for 2-MCP, 630 $\mu g.l^{-1}$ for 4-MCP, 290 $\mu g.l^{-1}$ for 2,4-DCP, 160 $\mu g.l^{-1}$ for 2,4,5-T3CP and 970 $\mu g.l^{-1}$ for 2,4,6-T3CP. These NOEC-values also show (similar to L(E)C50-values) a trend of increasing toxicity with increasing chlorination.

In a number of field studies, including ecosystem studies, adverse effects have been observed at PCP concentrations $\geq$ 34 $\mu g.l^{-1}$; concentrations up to 20 $\mu g.l^{-1}$ were without effect.

Toxicity to marine organisms

Data on marine organisms are very limited. Only for PCP there are a relatively large number of 48/96-hr L(E)C50-values. The (lowest) 48/96-hr L(E)C50-values from "single species" tests are 3,270 $\mu g.l^{-1}$ for 4-MCP, 1,700 $\mu g.l^{-1}$ for 2,4,5-T3CP, 1,900 $\mu g.l^{-1}$ for 2,3,5,6-T4CP and 53 $\mu g.l^{-1}$ for PCP.

Long-term tests (n = 4) with PCP have resulted in NOEC-values of 5 to 100 $\mu g.l^{-1}$.

Both the L(E)C50-values and NOEC-values reported here are similar to the respective toxicity values for freshwater organisms.

Table 2.1 Freshwater organisms - short-term toxicity tests with chlorophenols other than PCP: L(E)C50-values

Organism	A	Test-type	Test-water	pH	Hardness	Exp.-time	Crite-rion	Result µg/l	Reference
2-MCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	17,900*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	8,950	[1]
Daphnia magna	-	S-"closed"	art.	8.0	250	24-hr	L(E)C50	6,300	Kühn et al. '89b
Daphnia magna	-	S-"closed"	lake	-	-	48-hr	L(E)C50	7,400	Kopperman et al.,'74
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	**2,600**	LeBlanc '80 [2]
Pimephales promelas	+	F	lake	7.5	45	96-hr	LC50	12,000 α	Phipps et al.'81
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	13,775	Saarikoski & Viluksela,'82
3-MCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	15,800*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	7,900	[1]
4-MCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	8,100*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	4,050	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	3,400	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**2,500**	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	250	24-hr	L(E)C50	8,600	Kühn et al. '89b
Daphnia magna	-	S-"closed"	lake	-	-	48-hr	L(E)C50	4,800	Kopperman et al.'74
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	4,100	LeBlanc '80 [2]
Pimephales promelas	-	S-"closed"	lake	7.2-8.5	96-125	96-hr	LC50	≥ 3,800	Mayes et al., '83 [4]
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	8,490	Saarikoski & Viluksela,'82
2,3-DCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	5,200*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	2,600	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	4,100	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**3,100**	Kühn et al., '89a
2,4-DCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,700*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	1,350	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	2,500	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**1,400**	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	250	24-hr	L(E)C50	3,900	Kühn et al. '89b
Daphnia magna	-	S-"closed"	lake	-	-	48-hr	L(E)C50	2,600	Kopperman et al.,'74
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	2,600	LeBlanc '80 [2]
Pimephales promelas	+	F	lake	7.5	45	96-hr	LC50	8,200 α	Phipps et al.'81
Pimephales promelas	+	S	lake	7.5	45	48-hr	LC50	8,570 α	Phipps et al.'81
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	5,520	Saarikoski & Viluksela,'82
2,6-DCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	9,400*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	4,700	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	6,000	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**3,400**	Kühn et al., '89a
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	7,800	Saarikoski & Viluksela,'82

(to be continued)

Table 2.1 Freshwater organisms - short-term toxicity tests with chlorophenols other than PCP: L(E)C50-values (continued)

Organism	A	Test-type	Test-water	pH	Hardness	Exp.-time	Crite-rion	Result µg/l	Reference
3,4-DCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,800*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**1,400**	[1]
3,5-DCP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,100*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**1,050**	[1]
2,3,4-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,200*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**1,100**	[1]
2,3,5-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,300*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**1,150**	[1]
2,3,6-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	7,400*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**3,700**	[1]
2,4,5-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,100*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	1,050	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	1,500	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**900**	Kühn et al., '89a
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	2,700	LeBlanc '80 [2]
Pimephales promelas	-	F	lake	7.4-8.2	44-49	96-hr	LC50	1,270	Norberg-King, '89
Pimephales promelas	+	R	lake	7.4-8.2	44-49	96-hr	LC50	**900**	Norberg-King, '89
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	1,245	Saarikoski & Viluksela,'82
2,4,6-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	5,500*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	2,750	[1]
Daphnia magna	-	S-"closed"	art.	8.0	240	24-hr	L(E)C50	3,700	Kühn et al., '89a
Daphnia magna	-	S-"closed"	art.	8.0	240	48-hr	L(E)C50	**2,200**	Kühn et al., '89a
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	6,000	LeBlanc '80 [2]
Pimephales promelas	+	F	lake	7.5	43-48	96-hr	LC50	9,150 α	Phipps et al.'81
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	2,265	Saarikoski & Viluksela,'82
3,4,5-T3CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	900*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	**450**	[1]
2,3,4,5-T4CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	1,800*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	900	[1]
Pimephales promelas	+	F	lake	6.9-7.7	43-47	96-hr	LC50	440	Holcombe et al., '84
Salmo gairdneri	+	F	lake	6.9-7.7	43-47	96-hr	LC50	**205**	Holcombe et al., '84

(to be continued)

Table 2.1 Freshwater organisms - short-term toxicity tests with chlorophenols other than PCP: L(E)C50-values (continued)

Organism	A	Test-type	Test-water	pH	Hardness	Exp.-time	Criterion	Result µg/l	Reference
2,3,4,6-T4CP									
Daphnia magna	-	S-"closed"	well	7.4-9.4	173	48-hr	L(E)C50	**290**	LeBlanc '80 [2]
Poecilia reticulata	+	R	tap	7.0	80-100	96-hr	LC50	1,085	Saarikoski & Viluksela,'82
2,3,5,6-T4CP									
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	24-hr	L(E)C50	2,300*	Devillers & Chambon, '86
Daphnia magna	-	S-"closed"	art.	7.0-8.2	200	48-hr	L(E)C50	1,150	[1]
Daphnia magna	-	S	well	7.4-9.4	173	48-hr	L(E)C50	**570**	LeBlanc '80 [2]
"T4CP"									
Ceriodaphnia reticulata	-	S	lake	7.2-7.4	45	48-hr	L(E)C50	335	Mount & Norberg, '84 [3]
Daphnia magna	-	S	lake	7.2-7.4	45	48-hr	L(E)C50	405	Mount & Norberg, '84 [3]
Daphnia pulex	-	S	lake	7.2-7.4	45	48-hr	L(E)C50	1,000	Mount & Norberg, '84 [3]
Simocephalus vetulus	-	S	lake	7.2-7.4	45	48-hr	L(E)C50	145	Mount & Norberg, '84 [3]

art.: artificial test water

[1] Test compound: "analytical-grade" (purity > 95%). The 48-hr L(E)C50 (*) is not reported, but estimated from the 24-hr value using a factor of 2. This factor is based on the difference between the 24-hr and 48-hr L(E)C50 observe for a number of chlorophenols in the study by Kühn et al. (1989a) using the same test species.

[2] The range of pH-values is based on all tests that were conducted in this study, which included other compounds as well. Minimum purity of the test compounds was 80%.

[3] Test compound not specified.

[4] The value indicated is the lowest value observed in tests using different life stages.
Range pH-values and hardness: see [2].

Short-term L(E)C50-values of PCP are reviewed in the text.

Table 2.2 Freshwater organisms - long-term toxicity tests with chlorophenols: L(E)C50-values

Organism	A	Test-type	Purity comp.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result µg/l		Reference
2-MCP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	170,000	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	70,000	[5]	Shigeoka et al.,'88a
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	$LC50_g$	11,200	[3]	Könemann '79
Pimephales promelas	+	F	-	lake	7.5	45	8-d	LC50	6,300 α		Phipps et al.,'81
3-MCP											
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	29,000	[5]	Shigeoka et al.,'88a
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	$LC50_g$	6,400	[3]	Könemann '79
4-MCP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	29,000	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	38,000	[5]	Shigeoka et al.,'88a
2,3-DCP											
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	5,000	[5]	Shigeoka et al.,'88a
2,4-DCP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	9,200	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	14,000	[5]	Shigeoka et al.,'88a
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	$LC50_g$	4,200	[3]	Könemann '79
Pimephales promelas	+	F	-	lake	7.5	45	8-d	LC50	6,500 α		Phipps et al.,'81
2,6-DCP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	9,700	[5]	Shigeoka et al.,'88
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	29,000	[5]	Shigeoka et al.,'88a
3,4-DCP											
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	3,200	[5]	Shigeoka et al.,'88a
3,5-DCP											
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	2,300	[5]	Shigeoka et al.,'88a
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	$LC50_g$	2,700	[3]	Könemann '79
2,3,4-TCP											
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	2,000	[5]	Shigeoka et al.,'88a
2,3,5-TCP											
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	LC50	1,600	[3]	Könemann '79
2,3,6-TCP											
Astacus fluviatilis	-	R	-	tap	6.5	120	8-d	LC50	5,400		Kaila &
					7.5				19,000		Saarikoski, '77
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	LC50	5,100	[3]	Könemann '79
2,4,5-TCP											
Pimephales promelas	+	R	a_g	lake	7-8	45	7-d	LC50	740		Norberg-King, '89

(to be continued)

Table 2.2 Freshwater organisms - long-term toxicity tests with chlorophenols: L(E)C50-values (continued)

Organism	A	Test-type	Purity comp.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result µg/l		Reference
2,4,6-T3CP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	10,000	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	3,500	[5]	Shigeoka et al.,'88a
Pimephales promelas	+	F	-	lake	7.5	45	8-d	$LC50_g$	6,100 α		Phipps et al.,'81
3,4,5-T3CP											
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	LC50	1,100	[3]	Könemann '79
2,3,4,5-T4CP											
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	LC50	770	[3]	Könemann '79
2,3,4,6-T4CP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	10,100	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	1,300	[5]	Shigeoka et al.,'88a
2,3,5,6-T4CP											
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	LC50	1,400	[3]	Könemann '79
PCP											
Chlorella vulgaris	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	10,300	[5]	Shigeoka et al.,'88a
Selenastrum capricornutum	-	S_{closed}	a_g	art.	7.5	11	4-d	$EC50_g$	420	[5]	Shigeoka et al.,'88a
Astacus fluviatilis	-	R	a_g	tap	6.5	120	8-d	$LC50_g$	9,000		Kaila &
					7.5				53,000		Saarikoski, '77
Daphnia magna	-	R	a_g	lake	8.1	225	21-d	L(E)C50	**800**		Van Leeuwen et al.'87
Daphnia magna	+	R	-	art.	7.9	100	21-d	L(E)C50	435		
				art-50%			21-d	LC50	180	[4]	Adema '78
Brachydanio rerio	+	F	a_g	well	8.1	360	≥ 6-d	$LC50_{thr.}$	**994** α	[2]	Fogels & Sprague '77
Carassius auratus	+	F	a_g	-	7.6	148	14-d	$LC50_{thr.}$	**174** α	[1]	Cardwell et al.'76
Jordanella floridae	+	F	a_g	well	8.1	360	≥ 6-d	$LC50_{thr.}$	**1,600** α	[2]	Fogels & Sprague '77
Lepomis macrochirus	+	F	a_g	-	7.9	145	14-d	$LC50_{thr.}$	**198** α	[1]	Cardwell et al.'76
Pimephales promelas	+	F	a_g	-	7.8	156	14-d	LC50	**141** α	[1]	Cardwell et al.'76
Pimephales promelas	+	F	-	lake	7.4-8.4	45	8-d	$LC50_{thr.}$	210 α		Phipps et al.,'81
Poecilia reticulata	-	R	-	art.	7.3	25	≥ 7-d	$LC50_{thr.}$	**380**	[3]	Könemann, '79
Salmo gairdneri	+	F	t_g	-	8.1	360	≥ 6-d	$LC50_{thr.}$	**212** α	[2]	Fogels & Sprague '77
Salvelinus fontinalis	+	F	a_g	-	7.9	147	14-d	$LC50_{thr.}$	**109** α	[1]	Cardwell et al.'76

g = growth

Purity test compound: a_g = "analytical-grade" ("reagent-grade", "purified"); t_g = "technical-grade"

In most test with PCP, the test compound was added as NaPCP.

$LC50_{thr.}$: Median lethal threshold LC50 (incipient LC50).

Values for PCP which have been printed **bold** have been used in the "Kooijman (1987) extrapolation method" (see risk assessment).

[1] Test solutions prepared from (99% +) PCP. Test water was filtered through Whatman No.1 paper before measurement o NaPCP concentration. After 14-d of exposure the incipient LC50 (median lethal threshold) was reached in the tes with S. fontinalus. The fish were fasted during the acclimation (3 days) and exposure (14 days) period.

[2] Test solutions prepared from "technical-grade" NaPCP (79% +; 11% sodium salts of other chlorophenols; ≤ 10% iner clay). Fish were not fed during the acclimation (1 day) and exposure (≥ 6 days) period.

[3] Tests conducted in standard water according to Alabaster and Abram (1964); oxygen content ≥ 4 mg/l.

[4] Tests conducted in standard reference water, prepared according to Freeman, 1953.
In 100% and 50% SRW, control mortality was ≤ 8%; in 25% SRW (3-w LC50: 70 µg/l), control mortality was 30%.

[5] Continuous illumination. Growth measured by cell counting.

Table 2.3 Freshwater organisms - long-term toxicity tests with PCP: NOEC- and MATC-values

Organism	A	Test-type	Test-comp. & purity	Test-water.	pH	Hardness	Exp.-time	Criterion	Result µg PCP/l	Reference
Bacteria										
Pseudomonas fluorescens	-	S	PCP	n.m.	---	80	8-hr	$NOEC_g$	**1,000**	(lightning: none)
log-phase			≥ 99%				8-hr	$MATC_g$	1,800	(√ (1,000 x 3,200))
										Slooff & Canton '83 [7]
Algae										
Microcystus aeroginosa	-	S	PCP	n.m	7.8	25	4-d	$NOEC_g$	**1,000**	(lightning: continuous)
log-phase			≥ 99%				4-d	$MATC_g$	1,800	(√ (1,000 x 3,200))
										Slooff & Canton '83 [7]
Senedesmus pannonicus	-	S	PCP	n.m	7.7	54	4-d	$NOEC_g$	**100**	(lightning: continuous)
log-phase			≥ 99%				4-d	$MATC_g$	180	(√ (100 x 320))
										Slooff & Canton '83 [7]
Macrophytes										
Lemna minor "M 19"	-	S	PCP	n.m.	---	268	7-d	$NOEC_g$	**1,000**	(lightning: continuous)
2 fronts			≥ 99%				7-d	$MATC_g$	1,800	(√ (1,000 x 3,200))
										Slooff & Canton '83 [7]
Coelenterata										
Hydra oligactis	-	R	PCP	DSW	8.2	210	3-w	$NOEC_{s,g}$	**32**	
budless			≥ 99%				3-w	$MATC_{s,g}$	56	(√ (32 x 100))
										Slooff & Canton '83 [7]
Molluscs										
Lymnaea stagnalis	-	R	PCP	DSW	8.2	210				
			≥ 99%							
5-m old							6-w	$NOEC_{s,r}$	**10**	
eggs							7-d	$NOEC_h$	**3.2**	
								$MATC_{s,h,r}$	5.6	(√ (3.2 x 10))
										Slooff & Canton '83 [7]
Crustaceans										
Daphnia magna	+	R	PCP	SRW	7.9	100	3-w	$NOEC_{s,r}$	**180**	
P 1-d old --> F [lc]			-				3-w	$MATC_{s,r}$	240	(√ (180 x 320))
										Adema '78 [6]
Daphnia magna	-	R	PCP	DSW	8.2	210	3-w	$NOEC_{s,r}$	**100**	
P 1-d old --> F [lc]			≥ 99%				3-w	$MATC_{s,r}$	180	(√ (100 x 320))
										Slooff & Canton '83 [7]
Daphnia magna	+	F	PCP	lake	8.1	225	2-w	$NOEC_y$	**140**	α [5]
exponentially growing populations			97%							
Daphnia magna	-	R	PCP	lake	8.1	225	3-w	$NOEC_{s,g,r}$	**560**	
P < 1-d --> F [lc]			97%				3-w	$MATC_{s,g,r}$	750	(√ (560 x 1,000))
										Van Leeuwen et al.'87
Insects										
Culex pipiens	-	R	PCP	DSW	8.2	210	± 4-w	$NOEC_{s,d}$	**3,200**	
1st instar			≥ 99%				± 4-w	$MATC_{s,d}$	5,600	(√ (3,200 x 10,000))
										Slooff & Canton '83 [7]

(to be continued)

Table 2.3 Freshwater organisms - long-term toxicity tests with PCP: NOEC- and MATC-values (continued)

Organism	A	Test-type	Test-comp. & purity	Test-water.	pH	Hardness	Exp.-time	Criterion	Result µg PCP/l	Reference
Fish										
Oryzias latipes	-	R	PCP	DSW	8.2	210	± 6-w	$NOEC_{h,s,g,bh}$	**32**	
eggs --> | 4-w post-hatching [els]			≥ 99%				± 6-w	$MATC_{h,s,g,bh}$	56	(√ (32 x 100)) Slooff & Canton '83 [7]
Pimephales promelas	+	F		well	7.4	272				
fry < 2-w										
	PCP (purity 99%)						13-w	$NOEC_{s,g}$	**36** α	
							13-w	$MATC_{s,g}$	55	(√ (36 x 85)) α
	Dowicide EC-7 (91% PCP; "low-impurity")						13-w	$NOEC_{s,g}$	≥ **139** α	
	Composite of commercial PCP						13-w	$NOEC_{s,g}$	**6** α	
							13-w	$MATC_{s,g}$	9	(√ (6 x 13)) α Cleveland at al. '82
Pimephales promelas	+	F	PCP	well	7.4	272	13-w	$NOEC_{s,g,b-d}$	**66** α	
fry, 7-d			> 99% (ultrapurified)				13-w	$MATC_{s,g,b-d}$	93	(√ (66 x 130)) α Hamilton et al. '86 [3]
Pimephales promelas	+	F	PCP	lake	7-8	46	> 4-w	$NOEC_{e-d,s,g}$	**45** α	
eggs < 1-d --> 4-w post-hatching [els]			reagent-grade				> 4-w	$MATC_{e-d,s,g}$	57	(√ (45 x 73)) α Holcombe et al. '82
Poecilia reticulata	-	R	PCP	DSW	8.2	210	4-w	$NOEC_{s,g,bh}$	**100**	
3-4 w old			≥ 99%				4-w	$MATC_{s,g,bh}$	180	(√ (100 x 320)) Slooff & Canton '83 [7]
Salmo gairdneri	-	F	NaPCP	well	7.4	29	10-w	$NOEC_{s,g}$	**11**	
eggs < 1-d --> fry [els]			"purified"				10-w	$MATC_{s,g}$	14	(√ (11 x 19)) Dominguez & Chapman '84
Salmo gairdneri	+	F	NaPCP	lake	7.9	---				
eggs < 1-d --> 4-w of feeding [els]			> 99%				> 4-w	$NOEC_{e-d,s,g}$	**24** α	
							> 4-w	$MATC_{e-d,s,g}$	44 α	(√ (24 x 80)) Hodson & Blunt '81 [2]
Salmo gairdneri	+	F	PCP	tap	7.5	125	18-d	$NOEC_{o}$	**11** α (July)	
maturing females			> 99%				18-d	$MATC_{o}$	15	(√ (11 x 22)) α (July)
							18-d	$NOEC_{o}$	**50** α (December)	Nagler et al. '86
Salmo gairdneri	-	F	NaPCP	well	7.2	---	4-w	$NOEC_{s,g,f-c}$	**3**	
fry, 2.1-2.5 g			94%				4-w	$MATC_{s,g,f-c}$	5	(√ (3 x 8)) Matida et al.,'70 [1]
Salmo gairdneri	+	F	NaPCP	stream	7.8	---	> 9-w	$NOEC_{s,g}$	**8**	[4]
eggs < 1-d --> late alevins [els]			> 90%				> 9-w	$MATC_{s,g}$	11	(√ (8 x 16)) Chapman & Shumway '78
Amphibians										
Xenopus laevis	-	R	PCP	DSW	8.2	210	14-w	$NOEC_{s,d,g}$	**32**	
< 2-d old			≥ 99%				14-w	$MATC_{s,d,g}$	56	(√ (32 x 100)) Slooff & Canton '83 [7]

b-d = bone-development; bh = behaviour; d = development; e-d = egg-development; f-c = feed-consumption; g = growth
h = hatchability; o = oogenesis; r = reproduction; s = survival; y = yield

lc = life cycle test; els = early life stage test (egg-larval test)
n.m. = nutrient medium; DSW = Dutch Standard Water, representing Dutch surface water;
SRW = Standard Reference Water, representing U.S. surface waters
Fur further footnotes: see next page.

[1] Growth (both weight and lenght) was reduced about 30% at 8 µg/l; no statistical data available. Concentrations are nominal NaPCP concentrations.

[2] Exposure temperatures for eggs, alevins and fry were 5 or 10 ^{o}C, 5 or 15 ^{o}C and 11.7 ^{o}C, respectively. Biomass of fry exposed at a relatively high temperature of 20 ^{o}C was reduced at the lowest concentration tested (16 µg/l).

[3] Weight (but not length) was reduced significantly ($p \leq 0.05$) at 130 µg/l.

[4] NaPCP added as Santobrite® (containing > 90% NaPCP). Oxygen concentration 10 mg/l; at lower oxygen concentrations, increased mortality and reduced growth occured (for more details: see the text).

[5] At 140 µg/l, yield (mean number of daphnids) was reduced 10% ; the calculated EC50 was 230 µg/l.

[6] Composition standard reference water (SRW) according to Freeman '53.

[7] Purity PCP (≥ 99%): personal communication investigators.

Table 2.4 Freshwater organisms - long-term toxicity tests with chlorophenols other than PCP: NOEC- and MATC-values

Organism	A	Test-type	Purity test-comp.	Test-water	pH	Hardness	Exp.-time	Criterion	Result µg/l	Reference
2-MCP										
Crustaceans										
D. magna	+	R	-	SW	8.0	250	3-w	NOEC	**500**	[1,2]
P 1-d old --> F (lc)							3-w	$MATC_{s,r}$	700	(√ (500 x 1000)) Kühn et al. '89b
Fish										
Pimephales promelas	+	F	-	-	-	-	> 4-w	$NOEC_{h,s,g}$	4,000 α	[7]
eggs --> 4-w post hatching [els]							> 4-w	$MATC_{h,s,g}$	5,690	(√ (4,000 x 8,100)) Leblanc, '84b
4-MCP										
Crustaceans										
D. magna	+	R	-	SW	8.0	250	3-w	NOEC	**630**	[1]
P 1-d old --> F (lc)							3-w	$MATC_{s,r}$	900	(√ (630 x 1260)) Kühn et al. '89b
2,4-DCP										
Crustaceans										
Daphnia magna	+	R	-	SW	8.0	250	3-w	NOEC	320	[1,3]
P 1-d old --> F (lc)								$MATC_{s,r}$	450	(√ (320 x 640)) Kühn et al. '89b
Daphnia magna	+	R	a_g	lake	7.8	170	2-w	NOEC	780 α	
P neonates --> F (lc)							2-w	$MATC_{s,w,r}$	1,100	(√ (780 x 1,550)) Gersich & Millazo,'90
Fish										
Pimephales promelas	+	F	a_g	lake	7-8	46				
eggs < 1-d --> 4-w post-hatching [els]							> 4-w	$NOEC_{e-d,s,g}$	**290** α	
							> 4-w	$MATC_{e-d,s,g}$	365	(√ (290 x 460)) Holcombe et al. '8
2,4,5-T3CP										
Fish										
Pimephales promelas	+	R	a_g	lake	7-8	47	1-w	NOEC	360	[4]
larvae							1-w	$MATC_{s,g}$	495	(√ (360 x 685))
Pimephales promelas	-	-	-	-	-	-	4-w	$NOEC_{s,g}$	**160**	[5]
eggs --> post-hatching [els]							4-w	MATC	235	(√ (160 x 340)) Norberg-King '89
2,4,6-T3CP										
Fish										
Pimephales promelas	+	F	-	-	-	-	> 4-w	NOEC	**970** α	[7] Leblanc, '84b
eggs --> 4-w post hatching [els]							> 4-w	$MATC_{h,s,g}$	1,425	(√ (970 x 2,100))

e-d = egg-development; g = growth; h = hatchability; r = reproduction; s = survival; w = weight adults

lc = life cycle test; els: early life stage test (egg-larval test)

For further footnotes, see next page.

SW = Standard Water (according to DIN - German Instiitute of Standardization, 1982a,b)

a_g: "analytical-grade" ("reagent-grade")

[1] Test conducted in closed test vessels.
[2] Minimum concentration measured before renewal: 300 mg/l.
[3] Minimum concentration measured before renewal: 210 mg/l.
[4] Test conducted in sand and carbon filtered UV-sterilized lake water.
[5] Personal communication R. Spehar, fellow-worker of the Environmental Research Laboratory, Duluth, Minnesota.
[6] Test conducted in chlorinated lake water which was adjusted to hardness prior to autoclaving. In a similar 3-w test (Gersich and Milazzo, 1988, not evaluated), the resulting MATC was identical.
[7] Test conducted according to standard procedures (U.S. EPA, 1972).

Table 2.5 Marine organisms - short- and long-term toxicity tests with chlorophenols: miscellaneous toxicity values

Organism	A	Test-type	Purity test comp.	Test water	Salinity o/oo	Exp.-time	Criterion	Result µg/l	Reference
Short-term tests									
4-MCP									
Skeletonema costatum	-	-	-	-	-	-	L(E)C50	3,270	LeBlanc '84a
Mysidopsis bahia	-	-	-	-	-	-	L(E)C50	29,700	LeBlanc '84a
Cyprinodon variegatus	-	S	a_g	nsw	10-31	96-hr	LC50	5,400	Heitmuller et al.'81 [1]
2,4,5-T3CP									
Cyprinodon variegatus	-	S	a_g	nsw	10-31	96-hr	LC50	1,700	Heitmuller et al.'81 [1]
2,3,5,6-T4CP									
Cyprinodon variegatus	-	S	a_g	nsw	10-31	96-hr	LC50	1,900	Heitmuller et al.'81 [1]
PCP									
Rotifers									
Brachionus plicatilis	-	S	-	asw	15-30	24-hr	LC50	1,360	Snell & Persoone,'89
Oligochaetes									
Monopylephorus cuticulatus	-	R	-	-	20	96-hr	LC50	≥ 350	Chapman et al., 1982 [5]
Limnodriloides verrucosus	-	R	-	-	20	96-hr	LC50	≥ 65	Chapman et al., 1982 [5]
Polychaetes									
Ophryotrocha diadema	+	R	-	asw	33	96-hr	LC50	≥ 600	Hooftman & Vink, '80 [10]
Molluscs									
Crassostrea virginica	-	S	a_g	nsw	17	48-hr	EC50	40	Borthwick & Schimmel,'78 [3,8]
Crustaceans									
Palaemonetes pugio	+	F	a_g	nsw	18-31	96-hr	LC50	> 515 α	Schimmel et al.'78 [3]
Palaeomonetes pugio	-	S	a_g	nsw	24	96-hr	LC50	649	Borthwick & Schimmel,'78 [3]
Palaeomonetes pugio	-	R	a_g	nsw	10	96-hr	LC50	436	Conklin & Rao,'78 [3]
Penaeus aztecus	+	F	a_g	nsw	18-31	96-hr	LC50	> 195 α	Schimmel et al.'78 [3]
Fish									
Cyprinodon variegatus	+	F	-	nsw	24	96-hr	LC50	442 α	Parrish et al.'78 [2]
Cyprinodon variegatus	-	S	a_g	nsw	10	96-hr	LC50	≥ 223	Borthwick & Schimmel,'78 [6]
Fundulus similis	+	F	a_g	nsw	18-31	96-hr	LC50	> 306 α	Schimmel et al.'78 [3]
Lagodon rhomboides	+	F	a_g	nsw	18-31	96-hr	LC50	53 α	Schimmel et al.'78 [3]
Lagodon rhomboides	-	S	a_g	nsw	26	96-hr	LC50	38	Borthwick & Schimmel,'78 [3]
Mugil cephalus	+	F	a_g	nsw	18-31	96-hr	LC50	112 α	Schimmel et al.'78 [3]
Long-term tests									
PCP									
Polychaetes									
Arenicola cristata	+	S	t_g	nsw	22-24	6-d	NOLC	156	Rubinstein, '78 [9]
						6-d	$NOEC_f$	45 α	
						6-d	$MATC_f$	60	(√ (45 x 80))
Ophryotrocha diadema	+	R	-	asw	33	7-w	$NOEC^{s,g,r}$	5 α	Hooftman & Vink, '80 [7]
P 2-d old larvae --> F							$MATC^{s,g,r}$	7 α	(√ (5 x 11))

(to be continued)

Table 2.5 Marine organisms - short- and long-term toxicity tests with chlorophenols: miscellaneous toxicity values (continued)

Organism	A	Test-type	Purity test subst.	Test water.	Salinity o/oo	Exp.-time	Criterion	Result µg/l	Reference
Long-term tests - PCP (continued)									
Molluscs									
Crassostrea virginica	+	F	a_g	nsw	19-23	8-d	$EC50_g$	76 α	Schimmel et al. '78 [3,4]
Crustaceans									
Palaeomonetes pugio	-	R	a_g	nsw	10	9-w	$NOEC_{s,m-c}$	100	Conklin & Rao, '78 [3]
						9-w	$MATC_{s,m-c}$	223	(√ (100 x 500))
Fish									
Cyprinodon variegatus	+	F	-	nsw	24	5-m	$NOEC_{s,r,g}$	47 α	
P (eggs < 1-hr) -->						5-m	$MATC_{s,r,g}$	64 α	(√ (47 x 88))
F1 (4-w juveniles) [lc]									Parrish et al. '78 [2]

g = growth; r = reproduction; s = survival

asw = artificial sea water; nsw = natural sea water

a_g = "analytical-grade"; t_g = "technical-grade"

[1] Purity test compound: ≥ 80%.
[2] Purity test compound: "Baker-grade".
[3] Test compound added as NaPCP.
[4] Growth measured as shell deposition (mm shell/oyster).
[5] Values indicated are the lowest values derived at different environmental conditions (temperature 1-10 ^{0}C; pH 6-8)
[6] Value indicated is the lowest value derived from tests with different life stage (1-d to 6-w old fry).
[7] No statistics applied. The reproductive potential was reduced 32% and 54% at 11 and 33 µg/l, respectively. Exposure of adult worms did not affect reproduction at 11 µg/l.
[8] EC50 for abnormal embryonic development.
[9] Test compound "Dowicide G-ST" (79% NaPCP); concentrations measured 1 hour after introduction.
[10] Value indicated is that for larvae; adult worms were less sensitive.

Table 2.6 Relative toxicity of chlorophenols

Organism & Criterion	2-	3-	4-	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-	2,3,4-	2,3,5-	2,3,6,-	2,4,5-	2,4,6-	3,4,5-	2,3, 4,5-	2,3 4,6-	2,3 5,6-	PCP	Reference
Bacteria																				
Photobacterium phosphoreum (Microtox test)																				Kaiser et al., '84
30-min. EC50 (mg/l)	34	14	8.3	4.9	5.5	9.4	13	1.6	2.8	1.2	1.1	13	1.3	7.7	0.36	0.18	1.3	2.2	0.52	
RT:	0.01	0.04	0.06	0.1	0.09	0.05	0.04	0.3	0.2	0.4	0.5	0.04	0.4	0.07	1.4	2.9	0.4	0.23	1	
Pseudomonas																				Rübelt et al., 1982 [2]
EC (mg/l)	120	30	20	80	70	50	130	20	10	40	20	>500	20	170	--	12	--	--	60	
RT:	0.5	2.0	3.0	0.7	0.8	1.2	0.5	3.0	6.0	1.5	3.0	0.1	3.0	0.4		5.0			1	
Algae																				
Chlorella pyrenoidosa (fresh water)																				Huang & Gloyna, 1968 [7]
72-hr EC50 (mg/l)	150	50	75	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	7,5 µg/l	
RT	0.1x 10^{-4}	0.3x 10^{-4}	0.2x 10^{-4}		0.5x 10^{-4}								7.0x 10^{-4}	6.0x 10^{-4}					1	
Chlorella vulgaris (fresh water)																				Shigeoka et al., '88a
96-hr EC50 (mg/l)	170	--	29	--	9	--	10	--	--	--	--	--	--	10	--	--	10	--	10	
RT:	0.06		0.3		1.1		1							1			1		1	
Selenastrum capricornutum (fresh water)																				Shigeoka et al., '88a
96-hr EC50 (mg/l)	70	29	38	5	14	--	29	3.2	2.3	2.0	--	--	--	3.5	--	--	1.3	--	0.42	
RT:	<0.01	0.01	0.01	0.08	0.03		0.01	0.1	0.2	0.2				0.1			0.3		1	
Protozoa																				
Tetrahymena pyriformis																				Schulz et al., '86, '87
48-hr EC50	68	--	--	--	15	--	--	--	--	--	--	--	--	7.8	--	0.45	--	1.0	0.72	
RT:	0.01				0.05									0.09		1.6		0.7	1	
Crustaceans																				
Crangon septemspinosa (sea water)																				Kaiser et al., '84
96-hr LC50 (mg/l)	5.2	--	4.6	--	--	--	19.1	--	1.5	2.0	--	2.7	--	--	--	--	11.9	--	3.3	
RT:	0.6		0.7				0.2		2.2	1.6		1.2					0.3		1	

(to be continued)

Table 2.6 Relative toxicity of chlorophenols (continued)

Organism & Criterion													Reference					
2-	3-	4-	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-	2,3,4-	2,3,5-	2,3,6,-	2,4,5-	2,4,6-	3,4,5-	2,3, 4,5-	2,3 4,6-	2,3 5,6-	PCP
Crustaceans (continued)																		
Daphnia carinata (fresh water)													Shigeoka et al., 1988b [11]					
24-hr L(E)C50 (mg/l)																		
25	--	12	--	7	--	26	--	--	--	--	--	--	7.5	--	--	2.3	--	0.56
RT:																		
0.02		**0.05**		**0.08**		**0.02**							**0.07**			**0.24**		**1**
Daphnia magna (fresh water)													Shigeoka et al., 1988b [11]					
24-hr L(E)C50 (mg/l)																		
9.0	--	7.4	--	6.0	--	20.0	--	--	--	--	--	--	1.7	--	--	1.6	--	0.7
RT:																		
0.08		**0.09**		**0.12**		**0.04**							**0.4**			**0.4**		**1**
Daphnia magna (fresh water)													Devillers & Chambon, '86					
24-hr L(E)C50 (mg/l)																		
18	16	8.1	5.2	2.7	--	9.4	2.8	2.1	2.2	2.3	7.4	2.1	5.5	0.9	1.8	--	2.3	0.8
RT:																		
0.04	**0.05**	**0.1**	**0.15**	**0.3**		**0.09**	**0.3**	**0.4**	**0.4**	**0.3**	**0.1**	**0.4**	**0.15**	**0.9**	**0.4**		**0.3**	**1**
Daphnia magna (fresh water)													Kühn et al., 1989a					
48-hr L(E)C50 (mg/l)																		
--	--	2.5	3.1	1.4	--	3.4	--	--	--	--	--	0.9	2.2	--	--	--	--	0.5
RT:																		
		0.2	**0.2**	**0.4**		**0.1**						**0.5**	**0.2**					**1**
Daphnia magna (fresh water)													LeBlanc, 1980 [5]					
48-hr L(E)C50 (mg/l)																		
2.6	--	4.1	--	2.6	--	--	--	--	--	--	--	2.7	6.0	--	--	0.3	0.6	0.7
RT																		
0.3		**0.2**		**0.3**								**0.3**	**0.1**			**2.3**	**1.2**	**1**
Daphnia magna (fresh water)													Kopperman et al., 1974 [4]					
48-hr L(E)C50 (mg/l)																		
7.4	--	4.8	--	2.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Daphnia magna (fresh water)													Leblanc et al., '88 [9]					
7-d L(E)C50 (mg/l)																		
3.7	--	2.3	--	2.6	--	12.2	--	--	--	--	--	3.5	--	--	--	--	--	0.53
RT:																		
0.14		**0.23**		**0.20**		**0.04**						**0.15**						**1**
Daphnia magna (fresh water)													Shigeoka et al., 1988b [11]					
14-d NOEC survival/reproduction (mg/l)																		
0.08	--	0.4	--	0.3	--	1.0	--	--	--	--	--	--	0.65	--	--	0.65		0.36
RT:																		
4.5		**0.9**		**1.2**		**0.4**							**0.5**			**0.5**		

(to be continued)

Table 2.6 Relative toxicity of chlorophenols (continued)

Organism & Criterion	2-	3-	4-	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-	2,3,4-	2,3,5-	2,3,6,-	2,4,5-	2,4,6-	3,4,5-	2,3, 4,5-	2,3 4,6-	2,3 5,6-	PCP	Reference
Crustaceans (continued)																				
Daphnia pulex (fresh water)																				Shigeoka et al., 1988b [11]
24-hr L(E)C50 (mg/l)																				
	21	--	10	--	6.6	--	17	--	--	--	--	--	--	3.9	--	--	1.4	--	0.41	
RT:																				
	0.02		**0.04**		**0.06**		**0.02**							**0.1**			**0.3**		**1**	
Fish																				
Carassius auratus (fresh water)																				Kobayashi et al., '79
24-hr LC50 (mg/l)																				
	16.0	--	9.0	--	7.8	--	--	--	--	--	--	--	1.7	10.0	--	--	0.75	--	0.27	
RT:																				
	0.02		**0.03**		**0.03**								**0.16**	**0.03**			**0.36**		**1**	
Cyprinodon variegatus (sea water)																				Heitmuller et al., 1981
96-hr LC50 (mg/l)																				
	--	--	5.4	--	--	--	--	--	--	--	--	--	1.7	--	--	--	--	1.9	--	
"Fish" (species not reported)																				Ingols et al., 1966 [3]
24-hr LC50-value (mg/l)																				
	58	18	14	--	14	--	--	--	--	--	--	--	--	3.2	--	--	--	--	--	
Idus idus melanotus (fresh water)																				Rübelt et al., 1982 [1]
48-hr LC50 (mg/l):																				Krijgsheld & van der Gen, '86
	10.3	5.5	3.8	3.5	4.5	**2.8**	3.5	1.1	1.8	1.2	0.6	2.9	0.4	1.9	--	--	--	--	0.11	
	8.3	3	3				4						1	3		0.3	1		0.6	
RT:																				
	0.01	**0.02**	**0.03**	**0.03**	**0.02**	**0.04**	**0.03**	**0.10**	**0.06**	**0.09**	**0.20**	**0.04**	**0.27**	**0.06**					**1**	
Lepomis macrochirus (fresh water)																				Buccafusco, 1981 [6]
96-hr LC50 (mg/l)																				
	6.6	--	3.8	--	2.0	--	--	--	--	--	--	--	0.45	0.32	--	--	0.14	0.17	--	
Pimephales promelas (fresh water)																				Phipps et al., 1981
96-hr LC50 (mg/l):																				
	12	--	--	--	8	--	--	--	--	--	--	--	--	9	--	--	--	--	0.22	
RT:																				
	0.02				**0.03**									**0.02**					**1**	
192-hr LC50 (mg/l):																				
	6.3	--	--	--	6.5	--	--	--	--	--	--	--	--	6.1	--	--	--	--	0.21	
RT:																				
	0.03				**0.03**									**0.03**					**1**	

(to be continued)

Table 2.6 Relative toxicity of chlorophenols (continued)

Organism & Criterion	2-	3-	4-	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-	2,3,4-	2,3,5-	2,3,6,-	2,4,5-	2,4,6-	3,4,5-	2,3, 4,5-	2,3 4,6-	2,3 5,6-	PCP	Reference
Fish (continued)																				
Poecilia reticulata (fresh water)																				Saarikoski & Viluksela, '81,'82
96-hr LC50 at pH 8:	--	--	9.1	--	7.6	--	17.9	--	--	--	--	--	3.1	7.9	--	--	3.7	--	0.9	
RT:			**0.1**		**0.1**		**0.05**						**0.3**	**0.1**			**0.2**		**1**	
96-hr LC50 at pH 7:	13.8	--	8.5	--	5.5	--	7.8	--	--	--	--	--	1.2	2.3	--	--	1.1	--	0.44	
RT:	**0.03**		**0.05**		**0.08**		**0.06**						**0.4**	**0.2**			**0.4**		**1**	
96-hr LC50 at pH 6:	--	--	7.7	--	3.5	--	3.9	--	--	--	--	--	1.0	0.9	--	--	0.34	--	0.11	
RT:			**0.01**		**0.03**		**0.03**						**0.1**	**0.1**			**0.3**		**1**	
96-hr LC50 at pH 5:	--	--	6.3	--	--	--	--	--	--	--	--	--	--	0.6	--	--	--	--	0.04	
RT:			**<0.01**											**0.07**					**1**	
Poecilia reticulata (fresh water)																				Könemann, 1979 [4]
≥ 7-d LC50 (mg/l) at pH 7.8:	13.5	7.9	--	--	5.9	--	--	--	4.7	--	4.7	13.3	--	--	2.4	2.3	--	3.9	0.77	
RT:	**0.06**	**0.1**			**0.13**				**0.16**		**0.16**	**0.06**			**0.32**	**0.33**		**0.2**	**1**	
≥ 7-d LC50 (mg/l) at pH 7.3:	11.2	6.4	--	--	4.2	--	--	--	2.7	--	1.6	5.1	--	--	1.1	0.77	--	1.4	0.38	
RT:	**0.03**	**0.06**			**0.09**				**0.14**		**0.24**	**0.07**			**0.35**	**0.49**		**0.27**	**1**	
≥ 7-d LC50 (mg/l) at pH 6.1:	7.1	6.4	--	--	3.3	--	--	--	2.6	--	0.88	0.95	--	--	1.1	0.44	--	0.39	0.13	
RT:	**0.02**	**0.02**			**0.04**				**0.05**		**0.15**	**0.14**			**0.12**	**0.3**		**0.33**	**1**	
Salmo trutta																				Hattula et al., 1981a [8]
24-hr LC50 (mg/l)	--	--	--	--	1.7	--	4.0	--	--	--	0.8	--	0.9	1.1	--	--	0.5	--	0.2	
RT:					**0.12**		**0.05**				**0.25**		**0.22**	**0.18**			**0.4**		**1**	

RT: Relative toxicity (PCP = 1; chlorophenol "x": LE(C)50 PCP : L(E)C50 chlorophenol "x")

For further footnotes, see next page.

[1] Two sets of data have been listed by Rübeltt et al.(1982): for a number of compounds the data do not match. The data which are underlined, are from Krijgheld & van der Gen. Static test system; hardness test water 15^0 DH (± 250 mg/l, as $CaCO_3$), pH 7-8.

[2] Test conditions incompletely reported (for example: no data on exposure time, exposure concentrations, and test medium used). EC: concentration that reduced the number of cells significantly.

[3] Final dissolved oxygen concentration ≥ 5 mg/l. Test conditions very incompletely reported.

[4] From the data reported it is not clear whether the fish were exposed for 7 or 14 days.

[5] Minimum purity test compounds 80%. The total range of pH-values measured in these tests and tests with other compounds was 7.4 to 9.4.

[6] Minimum purity test compounds 80%. The total range of pH-values measured in these tests and tests with other compounds was 6.5 to 7.9. The total range of dissolved oxygen concentrations was 9.7 mg/l (at start) to 0.3 mg/l (after 96-hr).

[7] Study very poorly reported. Effect parameter: chlorophyll concentration. Extrapolated concentrations at which complete destruction of chlorophyll occured, are reported to be as follows: about 500 mg/l for monochlorophenols, 100 mg/l for 2,4-DCP, 10 mg/l for trichlorophenols, and 7.5 µg/l for PCP. RT: "Relative toxicity coefficient", based on the slope of the concentration-effect relationship. Concentrations that showed no substancial toxicity, were 10 mg/l for monochlorophenols and 1 mg/l for di- and trichlorophenols; a no-effect-concentration for PCP is not reported.

[8] No data on test medium.

[9] Artificial test water. The 7-d LC50-values were estimated from a combination of 2-d and 7-d toxicity data.

[10] Test medium: proteose peptone medium. Parameter: population growth.

[11] In Japanese.

List of abbreviations tables 2.1 to 2.6

A	+: Test substance analysed in test solution; -: Test substance not analysed in test solution, or: no data.
α	Value based on actual (measured) concentrations in test solutions, as mentioned explicitly in the literature source. Values <u>not</u> indicated by "α" are considered to be nominal concentrations.
-	Secundary literature source; primary source not available.
> and ≥	Value indicated is highest concentration used in the test.
< and ≤	Value indicated is lowest concentration used in the test.
Test type	S: static; R: renewal; F: flow-through (continuous flow).
Test time	hr: hour(s); w: week(s); m: month(s).
Criterion	LC50: Lethal concentration for 50% of the organisms exposed. EC50: Effect concentration for 50% of the organisms exposed. NOLC: No-observed-lethal-concentration. NOEC: No-observed-effect-concentration. MATC: Maximum-acceptable-toxicant-concentration: the theoretical threshold concentration between the highest concentration without effect (NOEC) and the lowest concentration with effect (EC). The MATC is calculated as follows: MATC = $\sqrt{(NOEC \times EC)}$.

The values which have been printed **bold** have been used in the risk assessment.

3 ECOTOXICITY-II: TERRESTRIAL ORGANISMS

3.1 ACCUMULATION

3.1.1 Plants (agricultural crops)

PCP

The uptake of PCP in soybean and spinach plants was studied in pot experiments in a greenhouse. The seeds were planted in a sterilized loamy sand soil (75% sand, 17% silt, 8% clay, 2% organic matter [OM)]) treated with a single application of 10 mg PCP.kg^{-1}. The soil in which soybean seeds were planted was inoculated with a suspension of *Rhizobium japonicum*. In soybean plants the highest PCP concentration in the stem was reached between day 8 and day 32, when the soil still contained 90% and 60%, respectively, of the amount of PCP added. At day 90, when PCP in soil had almost completely disappeared, the PCP level in the stem was 20% lower than that at day 32. Mature whole soybean plants harvested at day 90 contained 15 mg PCP.kg^{-1} fresh weight; the shoots, roots and seeds contained 5, 40 and 0.1 mg PCP.kg^{-1} fresh weight, respectively. Based on the afore-mentioned decrease in stem PCP concentration, maximum whole-plant PCP levels must have been (at least) 20 mg.kg^{-1} fresh weight. Whole spinach plants, harvested at day 64, contained 10 mg PCP.kg^{-1} fresh weight; shoots and roots contained 9 and 20 mg.kg^{-1} fresh weight, respectively. The levels of PCP metabolites identified in both plant species (tetrachlorophenols, tetrachloroanisole, tetrachloroanisoles) were very low, namely one to three orders of magnitude lower than that of PCP itself (Casterline et al., 1985).

Potatoes stored in PCP-treated wooden bins had elevated levels up to 2.7 mg.kg^{-1} and 0.5 mg.kg^{-1} of PCP and T4CP, respectively. Following a spraying program of cotton and soybeans with PCP (no further details reported), residue levels up to 2 mg.kg^{-1} were found (NRCC, 1982).

Miscellaneous chlorophenols

A limited survey for chlorophenols in food carried out in Canada before 1978 showed T4CP and PCP residues in carrots, turnips, cabbage, and beets in the low mg.kg^{-1} range, 1 to 8 mg.kg^{-1} (NRCC, 1982).

3.1.2 Earthworms - laboratory studies

Accumulation from the soil

Miscellaneous chlorophenols

The accumulation in earthworm species *Eisenia fetida* and *Lumbricus rubellus* of 3-MCP, 3,4-DCP, 2,4,5-T3CP, 2,3,4,5-T4CP and PCP was determined in a very humic sand (pH-KCl 5.6, 6.1% OM, 2.4% clay, CEC 10 meq.100 g^{-1}) and in a moderately humic sand (pH-KCl 5.2, 3.7% OM, 1.4% clay, CEC 7). The worms were exposed for 14 days to a nonlethal concentration (32-56 $mg.kg^{-1}$ dry weight), added to the soil as an aqueous solution in case of 3-MCP, 3,4-DCP and 2,4,5-T3CP and as a solid in case of 2,3,4,5-T4CP and PCP. After exposure, bioaccumulation factors (BCFs) were calculated by dividing the concentration in the worms ($mg.kg^{-1}$ dry weight; worms were analyzed after emptying their gut) by the average soil concentration ($mg.kg^{-1}$ dry weight) which was estimated from the 0-day and 14-day soil concentration on the basis of a first-order degradation rate. BCFs ranged from 0.5 to 8.5 (For 3-MCP, somewhat higher BCFs of 10 and 16 were reported in the two soils, but because of a relatively high degradation rate, these values are considered to be not reliable). Assuming 25% dry weight of worms, these BCFs are equivalent to 0.1 to 2, when based on wet weights of worms. The concentrations in the worms (measured by GLC with ECD) were not reported. In this study a fairly good correlation ($r = 0.86$ for *E. fetida* and $r = 0.70$ for *L. rubellus)* was found between Log BCF based on the concentration in soil solution (pore water) and the lipophilicity, expressed as Log P_{oct}; BCFs based on soil solution concentrations were within the range of 10 to 100 for 3-MCP and 3,4-DCP, of 10 to 500 for 2,4,5-T3CP and 2,3,4,5-T4CP, and of 500 to 1,000 for PCP (van Gestel et al., 1987; van Gestel and Ma, 1988).

PCP

The accumulation of [^{14}C]NaPCP in earthworm *[Allolobophora caliginosa]* was studied in an artificial loam soil (70% sand, 20% bentonite, 10% sphagnum peat, and ± 1% $CaCO_3$ to adjust pH to 6.6; the characteristics of this soil are very similar to the "OECD" artificial soil, see 3.2.3) containing 2.2 or 11.2 $mg.kg^{-1}$ dry weight. Earthworms were exposed to the soil 14 days after this was treated with test compound (equilibrium period), when ± 70% of the applied radioactivity was non-extractable. After 14 days of exposure, whole-body concentrations were 17 and 144 $mg.kg^{-1}$ (wet weight) at low and high exposure level, respectively. Of this amount, 15%-30% was

found in the gut contents. Whole-body (gut included) BCFs based on wet weights of worms and dry weights of soils were 8 and 13, respectively. Whole-body BCFs based on dry weights of both worms and soils were 37 and 50, respectively. Concentrations in worms after 14 days of exposure were about 50% higher than those after 7 days of exposure. Both concentrations in earthworms and soils are expressed as PCP-equivalents, based on radioactivity measurements (Haque and Ebing, 1988).

Accumulation from solution

PCP

In earthworms *[A. caliginosa,]* exposed to two concentrations (1 and 10 $mg.l^{-1}$) of [^{14}C]PCP or [^{14}C]NaPCP in aqueous solutions (pH-values not reported), over 90% of the radiolabel was absorbed after 6 hours of exposure, showing a rapid uptake. Concentrations of the respective equivalents after 24 hours of exposure were slightly lower than those after 6 hours. BCFs (calculated by dividing the concentration in the worms [$mg.kg^{-1}$ wet weight] by the initial concentration in the water [$mg.l^{-1}$]) after 6 hours of exposure were 2.4 to 3.6. The concentrations in worms were expressed as PCP-equivalents, based on radioactivity measurements. After exposure to [^{14}C]PCP and [^{14}C]NaPCP, $\pm$ 50% and $\geq$ 95% of the amount accumulated could be extracted from worms' tissues. After exposure to [^{14}C]PCP, up to 20% of the extractable amount was found as PCP, while after exposure to [^{14}C]NaPCP up to 50% of the extractable amount was found as PCP, indicating a difference in kinetics and metabolism. In both cases metabolites were polar compounds, the identity of which was not analyzed. After transfer of the worms to clean water or soil for 1 day, 1%-6% of the radiolabel was excreted. (Haque and Ebing, 1988).
Data on chlorophenols other than PCP are not available.

3.1.3 Earthworms and other invertebrates - field studies

PCP

The environmental fate and distribution of a single foliar application of [^{14}C]NaPCP at an equivalent rate of 5 $kg.ha^{-1}$ (equivalent to 8 $mg.kg^{-1}$ soil, dry weight, based on accumulation of the majority of the amount applied in the top 4 cm of the soil within the experimental time of 19 or 32 weeks) has been studied under outdoor conditions in lysimeters containing an urban terrestrial micro-ecosystem. The ecosystem consisted of

soil monoliths covered with plants and stocked with invertebrates, both herbivores and carnivores. The soil was a sandy loam (clay 2.9%, silt 13.7%, sand 83.4%, organic matter 2%, pH 6.8, CEC 8 meq.). After 3 weeks of exposure, the highest concentration (105 $mg.kg^{-1}$ wet weight) was found in springtails *Folsomia candia*, an insect species which feed mainly on organic debris. The next highest concentration (77 $mg.kg^{-1}$ wet weight) was found in harvestmen *Opiliones sp.* which are in general predatory but scavenging may be important. Concentrations in other invertebrates, including both herbivores and carnivores, ranged from 0.6 $mg.kg^{-1}$ wet weight in snails to 11 $mg.kg^{-1}$ wet weight in spiders. All concentrations are expressed as PCP-equivalents, based on radioactivity measurements. After about 3 weeks, the concentration in organisms appears to decrease, with the exception of that in snails. Whole-body BCFs (calculated on the basis of wet weights of organisms and litter, the main food source for detritophagous organisms) were $\leq$0.01 to 0.05 for most organisms, after 3 weeks of exposure; whole-body BCFs of 0.34 and 0.46 were calculated for harvestmen and springtails, respectively. If the concentrations in organisms are related to that in the top soil layer (0-1 cm: measured concentration $\pm$ 4 $mg.kg^{-1}$ dry weight), maximum BCFs reached values of 19 and 26 for harvestmen and springtails, respectively. According to the autors these values are not high enough to indicate potential danger to the organisms involved, with regard to ecological magnification. All BCFs were calculated on the basis of radioactivity measurements in both organisms and litter and/or soil. After 19 weeks, 0.35% and 0.02% of the radioactivity recovered, was found in earthworms and other invertebrates, respectively. After 32 weeks a similar result was found with regard to invertebrates other than earthworms; the activity found in earthworms at this pouint ot time is not reported (Haque et al., 1988).

Data on the accumulation of [^{14}C]NaPCP by two different earthworm species after 19 weeks of exposure in the outdoor lysimeters (see above) are reported by Haque and Ebing (1988). Whole-body concentrations were 11.5 $mg.kg^{-1}$ wet weight in *A. caliginosa* and 40 $mg.kg^{-1}$ wet weight in *L. terrestris*. Whole-body BCFs based on dry weights of both worms and soil were 30 and 100, respectively. Whole-body BCFs based on wet weights of the worms were 6 and 22, respectively. The concentration in soil after 19 weeks is reported to be 1.8 $mg.kg^{-1}$ dry weight; it is not reported to which part of the soil layer this concentration has been related. Both concentrations in earthworms and soil are expressed as PCP-equivalents, based on radioactivity measurements.

Data on chlorophenols other than PCP are not available.

3.2 TOXICITY

Most data on effects of chlorophenols on microbe-mediated processes, plants, and earthworms are summarized in the tables 3.1 to 3.4. In these tables, two values are listed for each result. Firstly, the experimentally determined toxicity value in the soil in question and secondly (according to Denneman and van Gestel, 1990 and Van der Meent et al., 1990), a calculated value which is an estimate of the toxicity value in a "standard soil" containing 10% organic matter. The calculated value is based on the assumption that the bioavailability, and hence, the toxicity, is directly and inversely proportional to the organic matter content of the soil.

3.2.1 Microbe-mediated processes - laboratory studies (table 3.1)

The available data, which refer to both short-term and long-term toxicity tests with PCP, are summarized in table 3.1. Data on chlorophenols other than PCP are not available.

3.2.2 Plants (agricultural crops) - laboratory studies (table 3.2)

Toxicity studies with miscellaneous chlorophenols (2-MCP, 3-MCP, 2,4-DCP, 3,5-DCP, 2,3,5-T3CP, 2,4,6-T3CP and PCP) resulting in EC50- and/or NOEC-values with regard to the parameter "growth inhibition" are summarized in table 3.2. In all tests, plants were exposed for 2 weeks.

Additional data

In pot experiments, treatment of a loamy sand soil (75% sand, 17% silt, 8% clay, 2% OM) with a single application of $\geq$ 20 mg PCP.kg^{-1} resulted in mortality of soybean and spinach plants. An application of 10 mg.kg^{-1} appeared to be without effect on survival and growth (Casterline et al., 1985). It is noted that growth was not measured quantitatively in this accumulation study.

3.2.3 Invertebrates - laboratory studies

Earthworms (table 3.3 and 3.4)

Toxicity tests with miscellaneous chlorophenols (3-MCP, 3,4-DCP, 2,4,5-T3CP, 2,4,6-T4CP, 2,3,4,5-T4CP and PCP) resulting in LC50-values are

summarized in table 3.3. In most tests, earthworms were exposed for 2 weeks.

In the comparative study by Van Gestel and Ma (1988, 1990), 2-w LC50-values for 3-MCP, 3,4-DCP, 2,4,5-T3CP, 2,3,4,5-T4CP and PCP were determined for *Eisenia andrei* and *Lumbricus rubellus* in four different soils. The LC50-values were calculated both on the basis of the concentrations in soil ($mg.kg^{-1}$ dry weight) and on the basis of the concentrations in soil pore water ($mg.l^{-1}$). In the former case the LC50-values were dependent on the soil used, with differences up to a factor of approximately 10 (considering each test compound and test species separately), see table 3.3. Further, toxicity appeared to be independent of chlorination. In the latter case the LC50-values in the different soils were largely independent on the soil used, with differences of a factor of 2 or less. This indicates that the toxicity is primarily dependent on the concentration in the soil pore water and, hence, can be predicted on the basis of the adsorption characteristics. Further, in the latter case there was a trend of increasing toxicity from 3-MCP towards PCP, although little difference in toxicity was observed between 2,4,5-T3CP, 2,3,4,5-T4CP and PCP (LC50-values for *E.andrei* and *L. rubellus* ranged from about 10 to 1 $mg.l^{-1}$ and from about 20 to 3 $mg.l^{-1}$, respectively; these ranges are based on data on all 5 compounds tested). For each compound tested, *E.andrei* was (somewhat) more sensitive than *L. rubellus;* this may be related to test temperature which was highest in tests with the former species.

Additional data

The lethal toxicity of PCP has been studied most extensively. In addition to the tests listed in table 3.3, PCP has been studied as a reference toxicant in two "ring tests" with *E andrei*. These tests were conducted in an artificial soil composed of 10% finely ground sphagnum peat, 20% kaolin clay, 69% fine sand and 1% calcium carbonate to adjust pH (pH 6.0 $\pm$ 0.5, 8% OM, 8% clay). The use of this artificial soil is recommended in the OECD (Organisation for Economic Co-operation and Development) guidelines for testing of chemicals. The one test (n = 18) resulted in an average LC50 of 75 ($\pm$ 41) $mg.kg^{-1}$ dry weight; the other test (n = 32) resulted in an average LC50 of 69 $mg.kg^{-1}$ dry weight (van Gestel and Ma, 1988; secundary source). The range of LC50-values is not reported.

Toxicity tests with PCP resulting in NOEC-values with respect to sublethal parameters (growth, reproduction) are summarized in table 3.4. The exposure time was 3-4 weeks.

NOEC-values for chlorophenols other than PCP are not available.

Additional data

Earthworm contact toxicity tests - miscellaneous chlorophenols

In a comparative study, the toxicity of 90 chemicals (mostly pesticides and pesticide derivates), including 2,4-DCP and 2,4,5-T3CP, was studied using a 48-hr contact toxicity test with earthworm *Eisenia fetida*. On the basis of their respective LC50-values the chemicals were classified into 5 categories, ranking from "supertoxic" (LC50 < 1 $\mu g.cm^{-2}$) to "relatively nontoxic" (LC50 > 1,000 $\mu g.cm^{-2}$). Both 2,4-DCP and 2,4,5-T3CP were found to be "extremely toxic" (LC50 1-10 $\mu g.cm^{2}$), and to be more toxic than the herbicides 2,4-D and 2,4,5-T from which they may be formed (Roberts and Dorough, 1984). In a modification of the 1984 OECD filter-paper contact test, a 48-hr LC50 of 1.8 μg PCP $.cm^{-2}$ was found, also for *E. fetida* (van Gestel and van Dis, 1988). On the basis of a comparison of contact tests and tests in soils using PCP and other compounds, the latter authors concluded that the former tests have no predictive value for the toxicity in soil, but only indicate the order of toxicity to be expected in soil.

Nematodes - mixed exposure to chlorophenols

In a preliminary experiment the effects of a mixture of chlorophenols on nematodes (roundworms) was studied. Columns packed by natural soils (2 podzolic sands, 1 eerd soil, and 1 marine clay) from 4 different locations in the Netherlands were sprinkled hourly during 7 months, with artificial rain containing a mixture of 3-MCP, 3,4-DCP, 2,3,5-T3CP, 2,3,4,6-T4CP and PCP. One of the podzolic sands was sprinkled with artificial rain without chlorophenols. Quantities of 3-MCP, 3,4-DCP, 2,3,5-T3CP, 2,3,4,6-T4CP and PCP extracted from the 0-2 cm layer of the chlorophenols-treated soils after 7 months were 0, 0-4, 13-48, 7-81 and 15-69 $\mu g.kg^{-1}$ dry weight, respectively. Quantities in the 2-4 cm layer were 0, 0-0.1, 0.6-14.2, 0.2-15.6 and 0.5-15.6 $\mu g.kg^{-1}$ dry weight, respectively. In lower layers the concentrations were negligible or could not be detected. After 7 months, all treatments (including sprinkling of rain without chlorophenols) had significantly affected the total number of nematodes in the 0-10 layer of all soils. The results of treatment with chlorophenols were not consistent in the different soils: treatment resulted in either an increase (2 soils) or a decrease (2 soils) in the total number of nematodes. This discrepancy is most possibly the result of the differences in species composition which existed in the soils at start. Classification of the nematodes into feeding groups indicates that treatment with chlorophenols resulted in a shift towards bacteriophagous nematodes. This

can be explained by an increase in the number of bacteria which use chlorophenols (and/or degradation products of these compounds) as food source. The shift towards bacteriophagous nematodes may induce corresponding changes in the food chain (Kappers and Wondergem-van Eijk, 1989).

3.2.4 Invertebrates - field studies

PCP

The effects of a single foliar application of [^{14}C]NaPCP at an equivalent rate of 5 kg.ha^{-1} (equivalent to 8 mg.kg^{-1} soil, based on accumulation of the majority of the amount applied in the top 4 cm of the soil within the experimental time of 19 or 32 weeks) has been studied under outdoor conditions in lysimeters containing an urban terrestrial micro-ecosystem. The ecosystem consisted of soil monoliths covered with plants and stocked with invertebrates, both herbivores and carnivores. Quantitative data on number of organisms are not reported, but according to the authors the treatment did not affect the arthropod density. After 19 weeks the concentration in this soil was 1.8 mg PCP.kg^{-1} dry weight; it is not reported to which part of the soil layer this concentration has been related. This concentration is expressed as PCP-equivalents, based on radioactivity measurements (Haque et al., 1988).
A field application of 12.5 kg PCP.ha^{-1} has reported to be toxic to earthworm species *L. terrestris* and *Allolobophora longa* (not available; cited in Van Gestel and Ma, 1988). Assuming a uniform distribution in the top 4 cm of the soil, this amount is equivalent to approximately 20 mg.kg^{-1} soil.
Field studies on chlorophenols other than PCP are not available.

Summary and conclusions "terrestrial organisms"

Accumulation

Most data available on the accumulation of chlorophenols in terrestrial organisms refer to earthworms.

Plants

In a study with 2 species of plants (spinach, soybean), whole-plant PCP concentrations of 10-15 mg.kg^{-1} fresh weight were measured 2 to 3 months after a single application of 10 mg PCP.kg^{-1} soil. The PCP concentrations

in roots were 2 to 8 times higher than those in shoots. Data on the accumulation in plants of chlorophenols other than PCP are not available.

Invertebrates

A laboratory study in which earthworms were exposed to sublethal concentrations (32-56 $mg.kg^{-1}$ dry weight) of 3-MCP, 3,4-DCP, 2,4,5-T3CP, 2,3,4,5-T4CP or PCP in soil, resulted in BCFs in the range of 0.1 to 2 (based on fresh weight of worms and concentrations of parent compound in soil). In this study a fairly good correlation was found between BCFs calculated on the basis of the concentrations in soil pore water and the lipophilicity of the compounds. BCFs based on soil solution concentrations were 10-100 for 3-MCP and 3,4-DCP, 10-500 for 2,4,5-T3CP and 2,3,4,5-T4CP, and 500-1,000 for PCP; these BCFs are similar to those reported for aquatic organisms. These data indicate that the accumulation is primarily dependent on the concentration in soil pore water. Another laboratory study with earthworms resulted in BCFs of 8 and 13, at soil PCP concentrations of 2 and 11 $mg.kg^{-1}$ dry weight. A field study resulted in BCFs of 6 and 22 for two different species of earthworms, at a soil PCP concentration of approximately 4 $mg.kg^{-1}$ dry weight; BCFs for other species of invertebrates usually were below 1, with maximum values of 19 and 26 for harvestman and springtails, respectively. The BCFs derived from the two last-mentioned studies are based on radioactivity measurements of PCP-equivalents, including both parent compound and metabolites.

It is concluded that chlorophenols can be concentrated from the soil by plants, earthworms and by some other terrestrial invertebrate species. The field study on the accumulation of PCP in diverse invertebrate species, including herbivores and carnivores, does not indicate a significant potential for biomagnification (accumulation in food chains) in invertebrates.

Toxicity

Laboratory studies

Laboratory studies resulting in L(E)C50- and/or NOEC-values are available for microbe-mediated processes, plants (agricultural crops) and, especially, earthworms. In the studies summarized below, the test compound was added to the soil. The toxicity values are expressed as $mg.kg^{-1}$ dry soil.

Microbe-mediated processes

A number of long-term (4 to 18 weeks) studies in which the effects of PCP on parameters such as mineralisation, nitrification, Fe(III)-reduction, heat output and ATP content was studied in different soils, resulted in NOEC-values of 2 to $\geq$ 20 $mg.kg^{-1}$.

Plants

In a comparative study with lettuce, 2-w EC50-values (parameter: growth) were 43 $mg.kg^{-1}$ for 2-MCP, 7 $mg.kg^{-1}$ for 3-MCP, 53 $mg.kg^{-1}$ for 2,4-DCP, 32 $mg.kg^{-1}$ for 3,5-DCP, 9 $mg.kg^{-1}$ for 2,3,5-T3CP, 16 $mg.kg^{-1}$ for 2,4,6-T3CP and 8 $mg.kg^{-1}$ for PCP. In a parallel study using the same soil, a somewhat lower 2-w EC50-value (3.2 $mg.kg^{-1}$) was found for PCP. Additional tests with PCP in another soil resulted in 2-w EC50-values of 4.8 and 57 $mg.kg^{-1}$ for lettuce and oats, respectively. For 2,3,5-T3CP and PCP, 2-w NOEC-values were 3.2 $mg.kg^{-1}$ (lettuce) and 0.32-10 $mg.kg^{-1}$ (lettuce, oats), respectively. NOEC-values for the other compounds are not available.

Earthworms

A comparative study in which the lethal toxicity of a number of chlorophenols was studied in "single species" tests with the earthworm species *Eisenia andrei* and *Lumbricus rubellus*, resulted in 2-w LC50-values of 79-633 $mg.kg^{-1}$ for 3-MCP, 134-680 $mg.kg^{-1}$ for 3,4-DCP, 46-875 $mg.kg^{-1}$ for 2,4,5-T3CP, 117-875 $mg.kg^{-1}$ for 2,3,4,5-T4CP and 84-4,627 $mg.kg^{-1}$ for PCP. The species *E. andrei* was consistently more sensitive than *L. rubellus*. Each compound was studied in 2 or 4 different soils, including the artificial soil recommended by the OECD. Considering each test compound and test species separately, the LC50-values were dependent on the soil used, with differences up to a factor of approximately 10. When the LC50-values were calculated on the basis of the concentrations in soil pore water, the values were largely independent of the soil used, indicating that the toxicity is primarily dependent on the concentration in soil solution and, hence, can be predicted on the basis of adsorption characteristics. In the latter case there was a trend of increasing toxicity from 3-MCP towards PCP, although little difference in toxicity was observed between 2,4,5-T3CP, 2,3,4,5-T4CP and PCP. In two other lethal toxicity studies with PCP, lower 2/4-w LC50-values were found, the lowest value being 15 $mg.kg^{-1}$ (NOLC: 10 $mg.kg^{-1}$). Tests with 2,4,6-T3CP in the artificial (OECD) soil resulted in 2-w LC50-values of 58-100 $mg.kg^{-1}$ for 4 different species.

Data on sublethal toxicity to earthworms are limited to 4 tests with PCP in

the artificial (OECD) soil. These tests, with *E. andrei*, resulted in NOEC-values of 5.6 to 20 $mg.kg^{-1}$, with respect to the parameters reproduction and/or growth. The exposure time in these tests was 3 to 4 weeks.

Field studies

A single field application of 5 $kg.ha^{-1}$ [^{14}C]NaPCP (equivalent to 8 $mg.kg^{-1}$ dry weight; top 4 cm) did not affect arthropod density. A field application of 12.5 kg $PCP.ha^{-1}$ (equivalent to 20 $mg.kg^{-1}$ dry weight; top 4 cm) has been reported to be toxic to earthworm species.

Table 3.1 Microbe-mediated processes - toxicity tests with PCP: miscellaneous toxicity values (laboratory studies)

Parameter	Soil	pH	%OM	%Clay	CEC	Temp.	Exp.-time	Criterion	Result in test soil (mg/kg dry weight)	Calculated* value in 10% OM soil (mg/kg dry weight)
"Bioactivity"	clay loam (peat)	5.9	21	31	-	16-22	18-w	NOEC	≥ 20 (ww)	9.5
"Bioactivity"	silt loam	6.9	2	25	-	16-22	18-w	NOEC	≥ 20 (ww)	100
"Bioactivity"	sandy loam	6.5	2	7	-	16-22	18-w	NOEC	2 (ww)	10
								MATC	6 (ww) ($\sqrt{(2 \times 20)}$)	30
							[1]	Zelles et al., '86		
ATP-content	agricultural	6.4	3	34	-	20	7-w	NOEC	2	6.7
Nitrification	sand	5.2	6	5	-	-	4-w	NOEC	11	18
Nitrification	loam	5.2	3	18	-	-	4-w	NOEC	12	40
Respiration	sand	5.2	6	5	-	-	5-hr	NOEC	≥ 1,370	≥ 2,280
Respiration	loam	5.2	3	18	-	-	5-hr	NOEC	125	417
H_2-oxidation	sandy loam	7	3	18	-	25	2-hr	EC50	177	590
								Denneman & van Gestel, '90#		
N_2-fixation	sandy loam	6.5	10	10	-	20	2-w	$EC50_{inh.}$	50	50
							[2]	Tam & Trevors, '81		
Mineralization of acetate	subsoil sands	6-7	<0.2	-	-	10	≤ 2-w	$EC50_{inh.}$	0.54 - 540	
							≤ 2-w	$NOEC_{inh.}$	0.18 - 180	
	surface sand	6-7	<0.2	-	-	10	≤ 2-w	$EC50_{inh.}$	45	
							≤ 2-w	$NOEC_{inh.}$	15	
							[3]	van Beelen et al., '89		

* For explanation, see "list of abbreviations tables 3.1 to 3.4"

\# Data evaluated by Denneman & van Gestel, RIVM.

[1] Three applications of 2 or 20 mg/kg soil (wet weight), at t = 0, 6, and 13 weeks; based on an estimated DT50 of 30 days, the concentration in soil ranged from ± 0.8-3.1 or 8-31, respectively.
Bioactivity parameters: ATP, heat output under both aerobic and anaerobic conditions and after amendment of soil with glucose, Fe(III)-reduction, and CO_2 production. Although all applications initially resulted in stimulation or inhibition of one or more of the parameters studied, most effects were reversible within 6 weeks.

[2] Test compound NaPCP, added to the non-sterile soil as aqueous solution; aerobic conditions. In both non-sterile soil under anaerobic condition and in sterilized soil with an addition of Azobacter sp., EC50-values were higher.

[3] Aerobic conditions. Different subsoils were used, from sandy aquifers, and one surface soil; the mineralization of a low acetate concentration (1 µg/l) was studied in slurries, at 2 times the water holding capacity.
The exposure-time in each test was 2 times the half-live of ^{14}C-acetate.
In one of the subsoils, the EC50- and NOEC-value under anaerobic conditions were 10 times lower than that under aerobic conditions. In the surface soil a clear stimulation was observed, even at the lowest concentration tested (1.5 mg/kg).
A toxicity value in 10% OM soil has not been calculated because of the very low percentage of OM in the test soils used and because of the divergent results in the different test soils used.

Table 3.2 Plants - toxicity tests with chlorophenols: EC50- and NOEC-values (laboratory studies)

Organism	Soil	pH	%OM	%Clay	Temperature. °C	Exp.-time	Criterion	Result in test soil (mg/kg dry weight)	Calculated* value in 10% OM soil (mg/kg dry weight)	
2-MCP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	43	215	
								Hulzebos et al., '89		[1]
3-MCP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	7	35	
								Hulzebos et al., '89		[1]
2,4-DCP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	53	265	
								Hulzebos et al., '89		[1]
3,5-DCP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	32	160	
								Hulzebos et al., '89		[1]
2,3,5-T3CP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	9	45	
								Hulzebos et al., '89		[1]
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	$NOEC_g$	3.2	16	
								Denneman & van Gestel, 90#		
2,4,6-T3CP										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	16	80	
								Hulzebos et al., '89		[1]
PCP										
Avena sativa	agricultural	-	5.7	<8	-	2-w	EC50	57	100	
						2-w	$NOEC_g$	10	17	
								Denneman & van Gestel, '90#		
Lactuca sativa	agricultural	-	5.7	<8	-	2-w	EC50	4.8	8.4	
						2-w	$NOEC_g$	1.0	1.7	
								Denneman & van Gestel, '90#		
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	$EC50_g$	8	40	
								Hulzebos et al., '89		[1]
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	3.2	16	
						2-w	$NOEC_g$	0.32	1.6	
								Denneman & van Gestel, '90#		

g = growth

* For explanation, see "list of abbreviations tables 3.1 to 3.4

\# Data evaluated by Denneman & van Gestel, RIVM.

Avena sativa = oats; Lactuca sativa = lettuce

[1] Soluble compounds were added to the soil as aqueous solution. Poorly soluble compounds were added as solid.

Table 3.3 Earthworms - toxicity tests with chlorophenols: LC50-values (laboratory studies)

Organism	Soil	pH	%OM	%Clay	CEC	Temp. °C	Exp.-time	Criterion	Result in test soil (mg/kg dry weight)	Calculated* value in 10% OM soil
3-MCP										
Eisenia andrei	mod. humic sand	5.2	3.7	1.4	6.6	23	2-w	LC50	79	213
	very humic sand	5.6	6.1	2.4	10.0	23	2-w	LC50	134	220
	art.(OECD) soil	6	8	8		23	2-w	LC50	130	162
	peaty soil	4	15.6	9		23	2-w	LC50	423	271
Lumbricus rubellus	mod. humic sand	5.2	3.7	1.4	6.6	15	2-w	LC50	140	378
	very humic sand	5.6	6.1	2.4	10.0	15	2-w	LC50	342	561
	art.(OECD) soil	6	8	8		15	2-w	LC50	247	309
	peaty soil	4	15.6	9		15	2-w	LC50	633	406
									[1] van Gestel & Ma '88, '90	
3,4-DCP										
Eisenia andrei	mod. humic sand	5.2	3.7	1.4	6.6	23	2-w	LC50	134	362
	very humic sand	5.6	6.1	2.4	10.0	23	2-w	LC50	240	393
	art.(OECD) soil	6	8	8		23	2-w	LC50	177	221
	peaty soil	4	15.6	9		23	2-w	LC50	423	271
Lumbricus rubellus	mod. humic sand	5	3.7	1.4	6.6	15	2-w	LC50	352	951
	very humic sand	5.6	6.1	2.4	10.0	15	2-w	LC50	486	797
	art.(OECD) soil	6	8	8		15	2-w	LC50	322	402
	peaty soil	4	15.6	9		15	2-w	LC50	680	436
									[1] van Gestel & Ma '88, '90	
2,4,5-T3CP										
Eisenia andrei	mod. humic sand	5.2	3.7	1.4	6.6	23	2-w	LC50	46	124
	very humic sand	5.6	6.1	2.4	10.0	23	2-w	LC50	76	125
	art.(OECD) soil	6	8	8		23	2-w	LC50	63	79
	peaty soil	4	15.6	9		23	2-w	LC50	165	106
Lumbricus rubellus	mod. humic sand	5.2	3.7	1.4	6.6	15	2-w	LC50	235	635
	very humic sand	5.6	6.1	2.4	10.0	15	2-w	LC50	316	518
	art.(OECD) soil	6	8	8		15	2-w	LC50	362	452
	peaty soil	4	15.6	9		15	2-w	LC50	875	561
									[1] van Gestel & Ma '88, '90	
2,4,6-T3CP										
Allolobophora tuberculata	art.(OECD) soil	6	8	8		20	2-w	LC50	108	135
Eisenia fetida	art.(OECD) soil	6	8	8		20	2-w	LC50	58	72
Eudrilus eugeniae	art.(OECD) soil	6	8	8		20	2-w	LC50	85	106
Perionyx excavatus	art.(OECD) soil	6	8	8		20	2-w	LC50	78	97
									[3] Neuhauser et al., '86	

(to be continued)

Table 3.3 Earthworms - toxicity tests with chlorophenols: LC50-values (laboratory studies) (continued)

Organism	Soil	pH	%OM	%Clay	CEC	Temp. °C	Exp.-time	Criterion	Result in test soil (mg/kg dry weight)	Calculated* value in 10% OM soil (mg/kg dry weight)
2,3,4,5-T4CP										
Eisenia andrei	mod. humic sand	5.2	3.7	1.4	6.6	23	2-w	LC50	117	316
	very humic sand	5.6	6.1	2.4	10.0	23	2-w	LC50	166	272
Lumbricus rubellus	mod. humic sand	5	3.7	1.4	6.6	15	2-w	LC50	515	1,392
	very humic sand	5.6	6.1	2.4	10.0	15	2-w	LC50	875	1,434
									[1] van Gestel & Ma '88, '90	
PCP										
Eisenia andrei	mod. humic sand	5.2	3.7	1.4	6.6	23	2-w	LC50	84	227
	very humic sand	5.6	6.1	2.4	10.0	23	2-w	LC50	142	233
	art.(OECD) soil	6	8	8		23	2-w	LC50	86	107
	peaty soil	4	15.6	9		23	2-w	LC50	503	322
Lumbricus rubellus	mod. humic sand	5	3.7	1.4	6.6	15	2-w	LC50	1,206	3,259
	very humic sand	5.6	6.1	2.4	10.0	15	2-w	LC50	1,013	1,661
	art.(OECD) soil	6	8	8		15	2-w	LC50	362	452
	peaty soil	4	15.6	9		15	2-w	LC50	4,627	2,966
									[1] van Gestel & Ma '88, '90	
Eisenia andrei	art.(OECD) soil	6	8	8	10.8	23	2-w	LC50	28	35
	sandy soil;	4.1	1.7	4.3	-	23	2-w	LC50	52	306
	sandy soil;	7	1.7	4.3	5.5	23	2-w	LC50	16	94
									[2] van Gestel & Van Dis '88	
Eisenia andrei	art. soil	7	10	5		22	4-w	LC50	87	87
									Denneman & van Gestel, '90#	
Eisenia fetida	art.(OECD) soil	6	8	8			2-w	LC50	50	62
							4-w	LC50	15	19
							4-w	NOLC	10	12
									Denneman & van Gestel, '90#	
Eisenia fetida	art.(OECD) soil	6	8	8			4-w	LC50	10	12
									van de Meent et al., '90#	
Enchytraeus albidus	art.(OECD) soil	6	8	8		12	4-w	LC50	136	170
									Denneman & van Gestel, '90#	

* For explanation, see "list of abbreviations tables 3.1 to 3.4"

\# Data evaluated by Denneman & van Gestel or by Van de Meent et al., RIVM.

[1] Purity test compounds ≥ 95%. E. andrei: laboratory culture. L. rubellus: collected in the field. The compounds 3-MCP, 3,4-DCP and 2,4,5-T3CP were dissolved in water plus some ethanol and added to the soils as aqueous solution. The compounds 2,3,4,5-T4CP and PCP were added to the soils as solid. In the two publications, different methods to calculate the LC50 were used; the results of the latter publication are listed in this table.

[2] Purity ≥ 95%; compound was added to the soils as a solid. The parent pH of the sandy soil was 4.1.

[3] Purity ≥ 98%; compound was added to the soil as aqueous solution (with some acetone or chloroform).

Table 3.4 Earthworms - toxicity tests with PCP: NOEC- and MATC-values (laboratory studies)

Organism	Soil	pH	%OM	%Clay	CEC	Temp.	Exp.-time	Criterion	Result in test soil (mg/kg dry weight)	Calculated* value in 10% OM soil (mg/kg dry weight)
Eisenia andrei clitellated adults	art.(OECD) soil	6	8	8		20	3-w	$NOEC_{s,r}$	10	12.5
							3-w	$MATC_{s,r}$	18 (√ (10 x 32))	22.5
									[1] van Gestel et al.'88	
Eisenia andrei clitellated adults	art.(OECD) soil	6	8	8		18-23	3-w ?	$NOEC_{g,r}$	20	25
							3-w ?	$MATC_{g,r}$	28 (√ (20 x 40))	35
									[2] Posthuma, '88	
Eisenia fetida	art.(OECD) soil	6	8	8			4-w	$NOEC_{r}$	9	11.2
							4-w	$NOEC_{a}$	5	6.2
									Denneman & van Gestel, '90#	
Eisenia fetida	art.(OECD) soil	6	8	8			4-w	$NOEC_{r}$	5.6	7
									van de Meent et al., '90#	

a = appearance; g = growth; r = reproduction; s = survival

* For explanation, see "list of abbreviations tables 3.1 to 3.4"

\# Data evaluated by Denneman & van Gestel or by Van de Meent et al., RIVM.

[1] Purity > 95%. PCP was added to the soil as solid. Before exposure, worms were pre-conditioned for one week. After 3 weeks of exposure, the cocoons produced were collected and incubated in untreated soil (slightly modified OECD soil) to establish hatchability. Parameters reproduction: cocoon production and hatchability.

[2] Before exposure, the worms were pre-conditioned for one week. After exposure of the worms (3 weeks ?) the cocoons were collected and incubated in either untreated or treated soil for 5 weeks. Parameters reproduction: cocoon production and number of juveniles per fertile cocoon. The parameter hatchability could not be evaluated because of inconsistent data. At 60 mg/kg dw a significant increase in weight of the worms was found.

List of abbreviations Tables 3.1 to 3.4

> and ≥	Value indicated is highest concentration used in the test.
Test time	hr: hour(s); w: week(s); m: month(s).
Criterion	LC50: Lethal concentration for 50% of the organisms exposed.
	EC50: Effect concentration for 50% of the organisms exposed.
	NOLC: No-observed-lethal-concentration.
	NOEC: No-observed-effect-concentration.
	MATC: Maximum-acceptable-toxicant-concentration: the theoretical threshold concentration between the highest concentration without effect (NOEC) and the lowest concentration with effect (EC). The MATC is calculated as follows: MATC = {√ (NOEC x EC)}.

Soil characteristics:

CEC: cation exchange capacity, expressed as meq.100 g^{-1}
OM: organic matter
dw: dry weight
ww: wet weight

OECD artificial soil:

10% sphagnum peat, 20% kaolin clay, 69% fine sand,
1% calcium carbonate to adjust pH.
The indicated soil characteristics are based on measurements in several tests.

* Calculated value in 10% OM soil = Experimental value x $\frac{10}{\%\ \text{OM-t}}$

% OM-t = % organic matter in test soil.

The calculated values which have been printed **bold** have been used in the risk assessment.

4 TOXICITY TO LIVESTOCK

4.1 CHEMOBIOKINETICS AND METABOLISM

All data in this section refer to oral studies with PCP. Most studies reported herein were focussed on accumulation.

4.1.1 Poultry

In chickens exposed for 8 weeks to "purified" PCP at dose levels of 0, 1, 10, 100 or 1,000 $mg.kg^{-1}$ feed, the highest PCP levels were measured in kidneys, followed by liver, heart, muscles, gizzard and adipose tissue. At the highest dose level tested, PCP levels in kidneys, liver, muscles and adipose tissue were 34, 17, 5-7 and 2 $mg.kg^{-1}$, respectively. In the 5-w post-treatment period, PCP levels in tissues decreased to levels below 0.5 $mg.kg^{-1}$; the slowest elimination rate was observed in adipose tissue, resulting in an elimination half-life of 12 days (Stedman et al., 1980). In a second 8-w study with chickens, exposed to a 88%-purity PCP-formulation (containing 12% T4CP) at dose levels of 0, 600, 1,200 or 2,400 $mg.kg^{-1}$ feed, the highest PCP levels were also measured in kidneys or liver. In these organs, PCP levels increased from < 0.1 $mg.kg^{-1}$ to 180-240 $mg.kg^{-1}$ at the highest dose level tested. The PCP levels in muscles of exposed animals were below 5, 10 and 40 $mg.kg^{-1}$ at increasing dose levels, respectively. Kidneys and liver also contained the highest levels of T4CP, up to 4-5 $mg.kg^{-1}$ (Presscott et al., 1982).

4.1.2 Mammals

In pigs (6 animals per group) exposed by lactose capsules to daily oral doses of 0, 5, 10 or 15 $mg.kg^{-1}$ bw of "purified"-PCP for 30 days, PCP levels in blood increased from 0.8 to 70-80 $mg.l^{-1}$; the PCP levels in liver, kidneys and muscles increased from 0.2-0.6 $mg.kg^{-1}$ to 26-29, 22-27 and 7-9 $mg.kg^{-1}$ in liver, kidneys and muscles, respectively. The levels in tissues did not increase (or increased only slightly) with increasing dose level (Greichus et al., 1979).

In newborn calves (3 animals/group) exposed to 0, 2 or 20 $mg.kg^{-1}$ $bw.day^{-1}$ of either "analytical-grade" PCP or "technical-grade" PCP, in milk, steady-state serum PCP concentrations of about 40 and 100 $mg.l^{-1}$ were reached within 5 days of treatment in low-dosed and high-dosed animals, respectively, independent of compound tested. The serum PCP concentration in control animals was about 0.1 $mg.l^{-1}$. After 5 days of treatment the dose levels were reduced to 1 and 10 $mg.kg^{-1}$ $bw.day^{-1}$. After a total exposure time of 6 weeks, PCP levels in liver and kidneys were increased from $\leq$ 0.1 $mg.kg^{-1}$ to 1 and 4-5 $mg.kg^{-1}$ in low- and high-dosed animals, respectively. Lungs, thymus and lympnodes of high-dosed animals contained 3-4 $mg.kg^{-1}$ and muscle tissue 1-2 $mg.kg^{-1}$. The highest PCP level, 7 $mg.kg^{-1}$ was measured in the thymus of high-dosed animals exposed to "technical-grade" PCP, consistent with severe thymus athrophy. With exception hereof, PCP tissue levels were independent of compound tested. All levels reported are total PCP levels, including both unconjugated and conjugated PCP. The blood of low-dosed animals contained 60% and 40% of unconjugated PCP and conjugated PCP, respectively (Hughes et al., 1985).

In 3 lactating cows exposed by gelatin capsules to 20 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP for 10 days and then to 10 $mg.kg^{-1}$ $bw.day^{-1}$ for an additional 60 days, the PCP concentration in blood and milk rapidly increased from 0.05 $mg.kg^{-1}$ (similar to that in their alfalfa hay, grain and grass feed) to a steady-state level of about 40 and 4 $mg.kg^{-1}$, respectively. Steady-state levels in blood and milk were reached within 20 and 5 days, respectively. Samples of urine, faeces and milk collected on day 28 contained 225, 5 and 4 $mg.kg^{-1}$, respectively, showing that urine is the major route for PCP excretion. In the post-treatment period, PCP levels in blood and milk decreased to control levels within 10 days. *It is noted that the persistence of the impurities hexachlorobenzene and polychlorinated dibenzo-p-dioxins in blood and milk was much higher than that of PCP itself (Firestone et al., 1979).*

The chemobiokinetics and metabolism of a single oral dose of 0.1 mg ^{14}C-PCP.kg^{-1} bw were studied in a lactating dairy cow which had been fed 0.2 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP for 14 weeks prior to ^{14}C-PCP administration and for 4 days post-administration. Both absorption and elimination characteristics in serum showed first-order kinetics, resulting in half-lives of 4.3 and 43 hours, respectively. The PCP levels in serum and urine peaked at about 10 and 20 hours, respectively. In the 3-d post-treatment period, 75% of the dose was excreted in the urine. Faeces and milk were minor routes of elimination, each containing about 5% of the dose. The half-live for elimination in urine and milk were very similar: 40

and 42 hours, respectively. The highest ^{14}C activity at necropsy (4 days after dosing) was found in the liver, followed by kidneys, gall bladder and lungs. Total PCP steady-state concentrations (resulting from long-term exposure) in kidneys, liver, lungs and muscles were 1.8, 1.6, 1.0 and 0.4 $mg.kg^{-1}$, respectively. Total PCP steady-state concentrations in serum, urine and milk were 6.3, 7.0 and 1.0 $mg.l^{-1}$, respectively. In the 3-d post-treatment period, 20% of PCP in serum was conjugated. The percentage of conjugated PCP in urine increased from 15% shortly after dosing to about 65% in the remaining period. Possible metabolites (T4CP, T3CP, DCP, tetrachloro-p-hydroquinone, pentachloranisole) could not be detected in serum or urine (Kinzell et al., 1985).
Van Gelder (1978) reported a half-life of less than 2 days in cattle (not available; cited in McConnell et al., 1980).

4.2 TOXICITY

One study excepted, all data refer to oral studies with PCP. The remaining study refers to an oral study with 2,4,5-T3CP.

4.2.1 Experimental studies

PCP

Oral LD50-values of 120 and 140 $mg.kg^{-1}$ bw have been reported for sheep and calves, respectively (chapter 1, table 1.1).
In a pilot study in which 4 young pigs were exposed by lactose capsules to "purified"-PCP, a dose level of 30 $mg.kg^{-1}$ $bw.day^{-1}$ resulted in acute toxicosis after 7 days. Exposure of newborn calves to a dose level of 20 $mg.kg^{-1}$ $bw.day^{-1}$ of either "analytical-grade" PCP or "technical-grade" PCP, in milk, resulted in acute toxicosis after 5 days (Hughes et al., 1985).
Van Gelder (1978) reported that there were no clinical effects on cattle fed 5 mg "technical-grade" $PCP.kg^{-1}$ $bw.day^{-1}$ for 14 days (not available; cited in Firestone et al., 1979 and in Exon et al., 1984).

Exposure of 1-day old broiler chickens, 40 animals per group, to "purified" PCP (purity not reported; 23 ppm OCDD; other impurities not reported) at dose levels of 0, 1, 10, 100 and 1,000 $mg.kg^{-1}$ feed, resulted in significantly reduced body weights at 1 and 1,000 $mg.kg^{-1}$ feed, but not at 10 and 100 $mg.kg^{-1}$ feed, after 8 weeks of exposure. Organ weights

(expressed as percentage of body weight) were significantly affected at 100 $mg.kg^{-1}$ feed (increased weight of kidneys) and 1,000 $mg.kg^{-1}$ feed (increased weight of kidneys; decreased weights of liver, spleen, heart and gizzard). All dose levels resulted in diarrhoea which persisted throughout the study and in minor histological changes in the liver, namely some fatty changes and bile duct proliferation (Stedman et al., 1980).
In two other 8-w experiments with broiler chickens especially the effects of "purified"-PCP (purity 88%; T4CP 12%; < 0.8 ppm OCDD, 0.3 ppm HpCDD, < 1 ppb HCDD and < 1 ppb TCDD) on the immunocompetence was investigated. In these experiments, groups of 25 to 50 1-day old chickens were given dose levels of 0, 600, (1,200), or 2,400 $mg.kg^{-1}$ feed. In one of these experiments, decreased survival was observed at 2,400 $mg.kg^{-1}$ $bw.day^{-1}$; microscopic lesions were not observed in any group; the latter is in contrast with the results of the study by Stedman et al. (1980). At 600 $mg.kg^{-1}$ $bw.day^{-1}$, body weight was not affected, but organ weights expressed as percentage of body weight were significantly affected (increased kidney weight; decreased weight of spleen and bursa of Fabricius). At this dose level, all immunological parameters used to study humoral and cell-mediated immunity were similar to that in controls, with exception of the lymphoproliferative response to "concanavalin A". At 2,400 $mg.kg^{-1}$ there appeared to be an immunosuppressive effect, especially with regard to cell-mediated immunity, but most parameters studied were not affected (Prescott et al., 1982).

In a 30-d study in which groups of 6-w old pigs (6 animals per group) were exposed by lactose capsules to daily oral doses of 0, 5, 10 or 15 $mg.kg^{-1}$ bw of "purified"-PCP (impurities: 1-5% T4CP; total content of higher chlorinated PCDD and PCDF 6 ppm), overt signs of toxicosis were not observed and feed consumption, total weight gain and weight of kidneys were not affected either. At 10 and 15 $mg.kg^{-1}$ $bw.day^{-1}$, liver weights were significantly increased. Additionally, blood urea nitrogen values were increased and the numbers of lymphocytes were decreased at these dose levels, although the numbers of lymphocytes still were within the normal range. The change (percentage increase between day 0 and day 30) in total leucocytes, gamma globulin and IgG was statistically decreased at all dose levels tested, indicating immunosuppression. Histopathological examination of liver, kidneys, spleen, brain and muscle only showed a "nonspecific diffuse cloudy swelling of hepatocytes, characterized by enlarged cells which had a finely vacuolated cytoplasm. Sinusoids were decreased in some cases, presumably due to encroachment by enlarged hepatocytes" (Greichus et

al., 1979; Hillam and Greichus, 1983). From the data reported it is not clear whether these histological changes were observed in all dosed groups or only at 10 and 15 mg.kg^{-1} bw.day^{-1}.

In a study in which 3 lactating cows were exposed by gelatin capsules to 20 mg.kg^{-1} bw.day^{-1} of "technical-grade" PCP for 10 days and then to 10 mg.kg^{-1} bw.day^{-1} for an additional 60 days, no clinical evidence of toxicosis was observed during the 10-w treatment period or during the 23-w post-treatment period (Firestone et al., 1979). Toxicity parameters were not investigated in this study which was focused on accumulation characteristics.

The differential toxicity of "analytical-grade"-PCP (aPCP) and "technical-grade" PCP (tPCP) has been investigated in three comparative studies with cattle.

In the first study, newborn bull calves averaging 7 days of age were exposed for 6 weeks to either aPCP or tPCP, in milk. The former compound (purity 99%) contained 1% T4CP, 1.2 ppm OCDD, 1.8 ppm HpCDD and < 0.2 ppm HxCDD; the latter compound (purity 85%-90%) contained 4%-8% T4CP, 2%-6% lower chlorophenols, 1,000 ppm OCDD, 380 ppm HpCDD, 170 ppm HxCDD and 0.04 ppm TCDD. Groups of 3 animals were initially (first 5 days on study) exposed to 2 and 20 mg PCP.kg^{-1} bw.day^{-1}; in the remaining part of the study the dose levels were reduced to 1 and 10 mg.kg^{-1} bw.day^{-1}, because a dose level of 20 mg.kg^{-1} bw.day^{-1} resulted in acute toxicosis in one out of 3 calves exposed (regardless of compound tested), leaving 2 animals in high-dosed groups. Exposure to 10 mg aPCP.kg^{-1} bw.day^{-1} resulted initially in decreased body weight gain (probably caused by the initial exposure level of 20 mg.kg^{-1} bw.day) and, at termination, in decreased spleen (- 30%) and thymus (- 55%) weights and in a decreased uptake of p-aminohippurate by slices of renal tissue, indicative of an effect on active transport processes. A dose of 1 mg aPCP.kg^{-1} bw.day^{-1} was without effect. Exposure to 10 mg tPCP.kg^{-1} bw.day^{-1} resulted in decreased feed intake and body weight, decreased weights of spleen (- 50%) and thymus (- 85%), increased liver weight (+ 13%), histological changes in the thymus (depletion of lymphocytes) and in the Meibomian gland of the eyelid (sqamous metaplasia of the epithelial lining of the duct; duct dilatation and hyperkeratosis), decreased uptake of p-aminohippurate by renal slices, decreased triiodothyronine, T_3, and thyroxine, T_4, levels in serum (thyroid function), and effects on other serum clinical chemistry parameters (decreased levels of total protein and albumin, and increased γ-glutamyl transferase activity, indicative of hepatic injury). At 1 mg tPCP.kg^{-1}

bw.day^{-1}, thymus weight was decreased (- 40%) and liver weight was increased (+ 25%) (Hughes et al., 1985).

In the second study, yearling female heifers were exposed for 5 months to aPCP, tPCP and mixtures thereof, in feed. The former compound contained 30 ppm higher-chlorinated PCDD and < 20 ppm higher-chlorinated PCDF; the latter compound contained 15 ppm HxCDD, 410 ppm HpCDD, 1,500 ppm OCDD, 57 ppm HxCDF, 130 ppm HpCDF and 90 ppm OCDF. In this study, groups of 3 animals were exposed to a) 100% aPCP, b) 90% aPCP + 10% tPCP, c) 65% aPCP + 35% tPCP or d) 100% tPCP; a fifth group served as control. All treatment groups were initially (first 6 weeks on study) exposed to a dose level of 650 mg PCP.kg^{-1} feed, equal to 20 mg PCP.kg^{-1} bw.day^{-1}. In the remaining part of the study the PCP level in feed was reduced to 490 mg.kg^{-1} feed, equal to 15 mg.kg^{-1} bw.day^{-1}, because of reduced weight gain in all dosed animals.

Exposure to 100% aPCP resulted in minimal (adverse) effects, namely an effect on thyroid function (decrease in serum T_3 and T_4 concentrations), on hepatic mixed function oxidases (a 3-fold increase in aryl hydrocarbon hydroxylase [AHH] activity and a shift in the spectral characteristics of cytochrome P450) and further a decrease in absolute and relative weight of the thymus, and in an increase in the amount of smooth endoplasmic reticulum in the hepatocytes. Exposure to increasing amounts of tPCP resulted in a large number of dose-related adverse effects, including decreases in feed consumption, feed conversion efficiency and body weight, an increase in liver and lung weights, a decrease in thymus weight, effects on haematological parameters (decreases in packed cell volume, haemoglobin content and red blood cell count), effects on hepatic mixed function oxidases (aryl hydrocarbon hydroxylase and aminopyrine N-demethylase activities, cytochrome P450 content and spectral characteristics thereof), effects on serum chemistry (increase in γ-glutamyl transferase), and, possibly, an effect on cell-mediated immunity. Most striking gross lesion in 2 out of three animals exposed to 100% tPCP was a villous-like hyperplasia of the mucosa of the urinary bladder. Minimal hepatic lesions (hyperplasia of the mucosal lining of bile duct and/or gall bladder) were related with exposure to tPCP. Furthermore, a dose-related hyperkeratotic lesion was found in the Meibomian glands of the eyelid, and animals exposed to 100% tPCP showed skin lesions including hyperkeratosis. A number of the effects observed in this study is consistent with the presence of impurties, especially polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) which similarly cause these effects (McConnell et al., 1980).

In the third study, Forsell et al. (1981) fed combinations of aPCP and tPCP to lactating dairy cattle at levels of 0.2 $mg.kg^{-1}$ $bw.day^{-1}$ for 80 days followed by 2.0 $mg.kg^{-1}$ $bw.day^{-1}$ for 60 days. No clinical and immunological effects were observed. PCP blood levels in these animals were reported to be as high as 12.5 $mg.l^{-1}$. On the basis of this study, the NOAEL in cattle was estimated to be > 2 $mg.kg^{-1}$ $bw.day^{-1}$ for 160 days (not available; cited in Exon, 1984).

Exposure of lactating dairy cattle to 0.2 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP in feed for 11 weeks, followed by exposure to a dose level of 2 $mg.kg^{-1}$ $bw.day^{-1}$ for an additional 8 weeks did not significantly affect body weight and milk production; biologically significant effects on haematological and clinical chemistry parameters were not observed either. However, weights of liver, lungs, kidneys, and adrenals (all expressed as percentage of body weight) were significantly increased at termination. Additionally, gross and histopathological changes, particularly in the kidneys (e.g. chronic diffuse interstitial nephritis, and swollen or atrophied glomeruli) and the urinary bladder (thickening of the wall, subacute urocystitis) were observed in all 4 treated animals (Kinzell et al., 1981).

Chlorophenols other than PCP

In a very limited toxicity study, steers (2 animals per group; body weight 160-285 kg) were exposed to 0, 18, 54, or 160 $mg.kg^{-1}$ $bw.day^{-1}$ of either zinc 2,4,5-trichlorophenate or 2,4,5-trichlorophenyl acetate for 11 weeks, in feed. High-dosed animals were continued on treatment for an additional 11 weeks. Feed consumption, body weight gain and haematological parameters (haemoglobin content, packed red blood cell volume) were similar in all groups throughout the study. Gross examination showed no abnormalities (Anderson et al., 1949).

4.2.2 Cases of intoxications

Among cattle, cases of chronic intoxications ascibed to "technical-grade" PCP have resulted in respiratory difficulties, decreased milk production, skin lesions, increased incidences of persistent infections, liver and kidney damage, increased abortion rates and death. In a number of these cases, PCP blood levels did not exceed 2 $mg.l^{-1}$ (Exon, 1984).

Summary and conclusions "livestock"

Chemobiokinetics and metabolism

Limited studies in which cattle was exposed to oral doses of 0.1 to 10 mg PCP.kg^{-1} bw.day^{-1} show that PCP is absorbed and excreted rapidly, with half-lives of hours and days, respectively. Urine is the major route of elimination; about half the amount of PCP in the urine is conjugated; possible metabolites such as T4CP and tetrachloro-p-hydroquinone are not known in cattle. Faeces and milk are minor routes of elimination. These data indicate that major aspects of chemobiokinetics and metabolism of PCP are similar to those in laboratory animals and humans (chapter 1).
In oral studies with different animals (broiler chickens, pigs, cattle), exposed to elevated PCP levels, the highest PCP concentrations were usually observed in the liver and kidneys. Exposure of cattle to 0.1 mg PCP.kg^{-1} bw.day^{-1} for 14 weeks or to 10 mg.kg^{-1} bw.day^{-1} for 6 weeks resulted in PCP concentrations of about 2 and 4-5 mg.kg^{-1}, respectively, in these organs. The PCP concentration in muscles was 0.4 and 1-2 mg.kg^{-1}, respectively. Exposure of young pigs to 5-15 mg.kg^{-1} bw.day^{-1} for 4 weeks resulted in PCP concentrations of 22-29 mg.kg^{-1} in liver and kidneys and of 7-9 mg.kg^{-1} in muscles. In the latter study, the concentrations in the tissues did not increase (or increased only slightly) with increasing dose level, in contrast with studies with cattle.
Data on chlorophenols other than PCP are not available.

Toxicity

In (pilot) studies, oral exposure of newborn calves or young pigs to "analytical-grade" PCP at dose levels of 20 and 30 mg.kg^{-1} bw.day^{-1}, respectively, resulted in acute toxicosis within one week; dose levels of 10 and 15 mg.kg^{-1} bw.day^{-1} did not result in overt signs of toxicosis. It is noted that the animals were administered the dose in milk or capsules, in one or two daily treatments.
Comparative oral studies in which cattle was exposed to either "analytical-grade" PCP or "technical-grade" PCP show that the latter compound is considerably more toxic, consistent with the high content of impurities (especially PCDD and PCDF). Therefore, data on these compounds are discussed separately (see also chapter 1, section 1.2.3).

"Analytical-grade PCP" ("pure" PCP)

Exposure of pigs or cattle (newborn calves, heifers) to 10-15 $mg.kg^{-1}$ $bw.day^{-1}$ of "analytical-grade" PCP, for 4 weeks to 5 months, resulted in effects such as increased liver weight, decreased weights of spleen and/or thymus, a reduced thyroid function, and histological and biochemical changes in the liver (increase in the amount of smooth endoplasmatic reticulum, slight increase in aryl hydrocarbon hydroxylase activity). The study with pigs resulted in immunosuppression at 5 $mg.kg^{-1}$ $bw.day^{-1}$, the lowest dose level tested. The study with newborn calves resulted in a dose-without-effect of 1 $mg.kg^{-1}$ $bw.day^{-1}$. It is noted that this latter study was very extensive with regard to the number of toxicity parameters studied, but very limited with regard to the number of test animals (3 per group) and exposure time (6 weeks).

Exposure of chickens for 8 weeks to "purified" PCP at dose levels of 100-600 $mg.kg^{-1}$ feed resulted in an effect on organ weight(s); these and higher dose levels did not affect the immunocompetence.

"Technical-grade" PCP

Exposure of cattle (newborn calves, heifers) to 10-20 $mg.kg^{-1}$ $bw.day^{-1}$ of "technical-grade" PCP, for 6 weeks to 5 months, resulted in a large number of effects including decreased body weight, increased or decreased organ weights, effects on liver function (increased aryl hydrocarbon hydroxylase activity, effects on cytochrome P450) and effects on haematology (anaemia). Most striking lesions were observed in the urinary bladder (hyperplasia) and in the Meibomian glands of the eyelid (hyperkeratosis). In the 6-w study with newborn calves, liver weight was increased and thymus weight was decreased at 1 $mg.kg^{-1}$ $bw.day^{-1}$, the lowest dose level tested.

The effects of exposure of livestock to either "analytical-grade" PCP ("pure" PCP) or "technical-grade" PCP are similar to those observed in studies with laboratory animals (chapter 1), both with respect to the effects observed and with respect to effect levels.

Chronic intoxications ascribed to "technical-grade" PCP have resulted in a variety of effects, for example decreased milk production, persistent infections, liver and kidney damage, increased abortion rates and death.

Data on chlorophenols other than PCP are not available, with exception of a very limited toxicity study with 2,4,5-T3CP.

5 RISK ASSESSMENT

5.1 RISK ASSESSMENT FOR MAN

5.1.1 Oral exposure

For 2 out of the 19 chlorophenols there are sufficient data on genotoxicity, reproductive toxicity (including teratogenicity), and chronic toxicity (including carcinogenicity) to establish an acceptable daily intake. These compounds are 2,4-DCP and PCP.
For the chlorophenols remaining there are insufficient data.

2,4-DCP

There is no evidence for teratogenicity and carcinogenicity, and insufficient evidence for mutagenicity of this compound. In a reproduction study in which the progeny was exposed both pre- and postnatally, a dose level of 3 $mg.kg^{-1}$ $bw.day^{-1}$ of >99%-pure 2,4-DCP resulted in an effect on the immunocompetence of the progeny; a dose of 0.3 $mg.kg^{-1}$ $bw.day^{-1}$ was without effect. All semichronic and chronic toxicity studies in which other toxicity parameters were studied, resulted in higher no-observed-(adverse)-effect-levels [NO(A)ELs]. Extrapolation of the NO(A)EL of 0.3 $mg.kg^{-1}$ $bw.day^{-1}$ to an acceptable daily intake for humans at life-time exposure, results in a value of 0.003 $mg.kg^{-1}$ $bw.day^{-1}$, using a margin of safety of 100. Assuming an average weight of 60 kg for adults, this value is equivalent to a total daily intake of 0.18 mg 2,4-DCP.

PCP

There is no evidence for teratogenicity, and insufficient evidence for mutagenicity of this compound. There is insufficient evidence for carcinogenicity in experimental animals; human data on carcinogenicity are inadequate for evaluation.
Comparative studies with "pure" PCP and "technical-grade" PCP show that the latter compound is considerably more toxic than the former, consistent with the impurities, especially polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) which are present in technical PCP-formulations. Therefore, "pure"-PCP and "technical-grade" PCP are discussed separately. In the text below, "pure" PCP includes formulations (such as "Dowicide EC-7") with a total PCDF and PCDD content up to 30 ppm.

"Pure" PCP

Semichronic and chronic toxicity studies resulted in a NO(A)EL of 3 $mg.kg^{-1}$ $bw.day^{-1}$. Extrapolation of this NO(A)EL to an acceptable daily intake for humans at life-time exposure, results in a value of 0.03 $mg.kg^{-1}$ $bw.day^{-1}$, using a margin of safety of 100. Assuming an average weight of 60 kg for adults, this values is equivalent to a total daily intake of 1,8 mg PCP.

"Technical-grade" PCP

In semichronic toxicity studies, the lowest dose level tested (1 $mg.kg^{-1}$ $bw.day^{-1}$) resulted in histo(patho)logical liver changes and effects on the hepatic enzymes aryl hydrocarbon hydroxylase and glucuronyl transferase. In a reproduction study in which the progeny was exposed both pre- and postnatally, the lowest dose tested (0.25 $mg.kg^{-1}$ $bw.day^{-1}$) resulted in an effect on the immunocompetence of the progeny. Lower dose levels have not been tested. Therefore, and because of the variable composition of "technical-grade" PCP, an acceptable daily intake can not be establish.

5.1.2 Exposure by inhalation

Data on (no) effect levels at exposure by inhalation are very limited (PCP) or lacking (chlorophenols other than PCP). Therefore, acceptable airborne concentrations can not be established.

Limited animal studies show that an airborne PCP concentration of 3 $mg.m^{-3}$ can result in an increased liver weight and/or effects on biochemical parameters (exposure 4 hours per day, for 4 months). These and acute toxicity data indicate that PCP is (at least) 10 times more toxic at exposure by inhalation than at oral exposure.

Limited data on non-occupational exposure indicate that prolonged exposure to an (indoor) PCP level of 5 $\mu g.m^{-3}$ (range 2-10 $\mu g.m^{-3}$) does not result in measurable effects on general health status.

5.2 RISK ASSESSMENT FOR THE ENVIRONMENT

In this risk assessment, different extrapolation procedures have been used to establish acceptable concentrations ("maximally acceptable risk-levels", MTRs)) of chlorophenols in surface water and soil, on the basis of L(E)C50-values and/or NOEC-values from "single species" toxicity tests. MTRs are considered to be concentrations below which there is no unacceptable risk for ecosystems at long-term exposure, although sensitive species may be adversely affected.

The principles of these procedures are described in the "Integrated Criteria Document Chlorophenols". For more details the reader is referred to the original pubications on these procedures.

5.2.1 Aquatic organisms

Fresh water

The results of the extrapolation procedures and the toxicity values which have been used in these procedures are summarized in table 5.1 (PCP) and table 5.2 (chlorophenols other than PCP).

PCP (table 5.1)
In conformity with a recent report of the National Institute of Public Health and Environmental Protection ("RIVM", Van de Meent et al., 1990), a concentration of 2 $\mu g.l^{-1}$ ("modified" Van Straalen-procedure) is recommended as MTR for PCP in fresh surface water. This value is calculated on the basis of NOEC-values with regard to sublethal parameters (long-term studies).

Chlorophenols other than PCP (table 5.2)
For these compounds, NOEC-values with regard to sublethal parameters are very limited or lacking. Therefore, MTRs can not be calculated with the "modified" Van Straalen procedure which is used preferably. The available data clearly show, despite differences in toxicity of compounds within one group of isomers (e.g. dichlorophenols), that the toxicity of chlorophenols increases with increasing chlorination. Therefore, only one MTR for all individual compounds within each group of isomers has been derived, primarily based on the results of a "modified" EPA-procedure. This results in the following MTRs: 25 $\mu g.l^{-1}$ for all monochlorophenols, 15 $\mu g.l^{-1}$ for all dichlorophenols, 2.5 $\mu g.l^{-1}$ for all trichlorophenols and 1 $\mu g.l^{-1}$ for all tetrachlorophenols. Because of the limited number of toxicity values, these MTRs are considered to be indicative values.

Sea water

For the majority of the compounds, both L(E)C50- and NOEC-values are lacking. For the compounds remaining, the numbers of toxicity values are (very) limited. The available data indicate similar sensitivities of

freshwater and marine organisms for chlorophenols. Therefore, the (indicative) MTRs voor freshwater are also recommended for sea water.

5.2.2 Terrestrial organisms

For terrestrial organisms, the numbers of toxicity values are (very) limited. Therefore, only one extrapolation procedure has been used. The results of this procedure (a "modified" EPA-procedure) and the toxicity values which have been used in this procedure are summarized in table 5.3. The L(E)C50- and NOEC-values which are listed in this table have been converted from an experimental value into an estimated value in a "standard soil" containing 10% organic matter, to correct for differences in toxicity caused by the use of different test soils (see the equation in the footnote of table 5.3). Accordingly, the values calculated with the extrapolation procedure refer to a 10% OM standard soil.

PCP

For PCP there are both L(E)C50-values and (a limited) number of NOEC-values available. Extrapolation of the lowest L(E)C50 and NOEC results in calculated concentrations of 0.08 and 0.16 $mg.kg^{-1}$ dry weight, respectively. Because NOEC-values are used preferably to establish an acceptable concentration, a concentration of 0.2 $mg.kg^{-1}$ dry weight is recommended as indicative MTR for PCP in a 10% OM soil. For soils containing a percentage of OM other than 10%, MTRs can be calculated using the equation in table 5.3.

Chlorophenols other than PCP

For half of these compounds there is at least one L(E)50 available. Extrapolation of the lowest value for each compound results in calculated concentrations ranging from 0.04 to 0.72 $mg.kg^{-1}$ dry weight. In contrast with the toxicity values for aquatic organisms, those for terrestrial organisms do not show a clear trend of increasing toxicity with increasing chlorination, not even in idential studies (This may be the result of the fact that soluble compounds were usually added to the soil as aqueous solution whereas less soluble compounds were added as solid). Therefore, and because of the limited number of toxicity values available, a range of 0.1 tot 1 $mg.kg^{-1}$ dry weight is considered as indicative MTR, for individual compounds, in a 10% OM soil.

Table 5.1 Calculated "acceptable" concentrations (μg/l) of PCP in fresh water, based on extrapolation procedures according to Slooff et al. (1986), Kooijman (1987), Van Straalen (1989) and RIVM (Van de Meent et al, 1990).

Input			Result (μg/l)
NOEC-values (long-term tests; n = 26)			
Van Straalen[1]:	(n = 26)	---------->	1.2
RIVM[2]:	(n = 10)	---------->	**2.0** "MTR"
Input			
Lowest NOEC-value: 3 μg/l (long-term tests)			
Slooff et al.[3]:	(n = 1)	---------->	0.3
EPA[4]:	(n = 1)	---------->	0.3
Input			
L(E)C50-values ("long-term" tests)			
Kooijman:	(n = 9)	---------->	0.5

"MTR": "maximally acceptable risk-level" (see the text).

n = number of input data.

1 Original Van Straalen-procedure; input: all available NOEC-values).
2 "Modified" Van Straalen-procedure: a revised statistical technique has been used, and the NOEC-values are clustered according to selected taxonomical groups (input: 1 NOEC for each group selected)
3 $\text{Log NOEC}_{\text{ecosystems}}$ = [+0.63 + 0.85.Log NOEC] : 33.5 (uncertainty factor).
4 Assessment factor of 10.

Table 5.2 Calculated "acceptable" concentrations (μg/l) of chlorophenols other than PCP in fresh water, based on the extrapolation procedures according to Slooff et al. (1986) and RIVM (Van de Meent et al., 1990)

Compound	Input				Result (μg/l)				
	LOWEST L(E)C50 I	(n)	LOWEST NOEC II	(n)	SLOOFF et al. I[1]	II[2]	RIVM EPA-MODIFICATION I[3]	II[4]	"MTR"
2-	2600	(10)	500	(2)	1.9	25	26	50	25
3-	6400	(3)			4.0		64		25
4-	2500	(9)	630	(1)	1.9	30	25	63	25
2,3-	3100	(3)			2.2		3.1		15
2,4-	1400	(12)	290	(3)	1.2	16	14	29	15
2,5-	2800~	(0)			2.0		2.8		15
2,6-	3400*	(5)			2.4		34		15
3,4-	1400*	(2)			1.2		1.4		15
3,5-	1050*	(3)			0.9		10		15
2,3,4-	1100*	(2)			1.0		1.1		2.5
2,3,5-	1150*	(2)			1.0		1.1		2.5
2,3,6-	3700*	(3)			2.6		3.7		2.5
2,4,5-	900	(7)	160	(2)	0.8	10	0.9	16	2.5
2,4,6-	2200*	(8)	970	(1)	1.7	44	22	97	2.5
3,4,5-	450*	(2)			0.5		0.4		2.5
2,3,4,5-	205	(4)			0.2		0.2		1
2,3,4,6-	290	(4)			0.3		2.9		1
2,3,5,6-	570	(3)			0.6		0.6		1

"MRT": "maximally acceptable risk-level" (see the text).

(n) Number of available values from tests conducted according to current guidelines for aquatic toxicity testing (primary literature source available).
(L(E)50-values: table 2.1 en 2.2; NOEC-values: table 2.4).

* Estimated 48-hr L(E)C50-value for the water flea Daphnia magna (24-hr experimental value : factor of 2).

~ Primary literature source not available.

[1] $\text{Log NOEC}_{\text{ecosystems}}$ = [-0.55 + 0.81.Log L(E)C50] : 85.7 (uncertainty factor).

[2] $\text{Log NOEC}_{\text{ecosystems}}$ = [+0.63 + 0.85.Log NOEC] : 33.5 (uncertainty factor).

[3] An assessment factor of 100 is applied in case there is at least 1 "reliable" L(E)C50 voor each of the following taxonomic groups: algae, crustaceans and fish; in the cases remaining, an assessment factor of 1000 is applied.

[4] Assessment factor of 10.

Table 5.3 Calculated "acceptable" concentrations (mg/kg dry weight) of chlorophenols in soil, based on an extrapolation procedure according to "EPA".

Compound	L(E)C50-values			Result[1] (mg/kg dry weight)
	groups	number of values	lowest value *	
2-	p	1	215	0.21
3-	p,e	1,8	35	0.35
2,4-	p	1	265	0.26
3,4-	e	8	221	0.22
3,5-	p	1	160	0.16
2,3,5-	p	1	45	0.04
2,4,5-	e	8	79	0.08
2,4,6-	p,e	1,4	72	0.72
2,3,4,5-	e	4	272	0.27
PCP	p,e,m-a	3,15,2	8	0.08
	NOEC-values			Result[2] (mg/kg dry weight)
	groups	number of values	lowest value *	
2,3,5-	p	1	16	1.6
PCP	p,r,m-a	4,4,6	1.6	0.16

e = earthworms; p = plants; m-a = microbial activities

* The experimental values (V_e) have been converted into estimated values (V_s) into a "standard soil" containing 10% organic matter (% OM-s: 10%), on the basis of the percentage of organic matter in the test soil (% OM-t), using the following equation:

$$V_s = V_e \times \frac{10}{\%\ OM\text{-}t}.$$

In most tests with plants, the % OM in the test soil was 1.4%; in these cases a percentage of 2. (% OM-t = 2) has been used in the equation .

[1] An assessment factor of 100 is applied in case there is at least 1 L(E)C50 for each of the following groups: plants (p) and earthworms (e); in the cases remaining an assessment factor of 1000 is applied.

[2] Assessment factor of 10.

REFERENCES

Adema, D.M.M. (1978)
Daphnia magna as a test animal in acute and chronic toxicity tests
Hydrobiologia 59, 125-134

Ahlborg, U.G. and K. Larsson (1978)
Metabolism of tetrachlorophenol in the rat
Arch. Toxicol. 40, 63-74

Ahlborg, U.G. and T.M. Thunberg (1978)
Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the *in vivo* and *in vitro* dechlorination of pentachlorophenol
Arch. Toxicol. 40, 55-61

Ahlborg, U.G. and T.M. Thunberg (1980)
Chlorinated phenols: occurence, toxicity, metabolism, and environmental impact
CRC Crit. Rev. Toxicol. 7, 1-27

Anderson, G.W. et al. (1949)
Cattle-feeding trials with derivatives of 2,4,5-trichlorophenol
J. Am. Vet. Med. Assoc. 115, 121-123

Anderson, K.J. et al. (1972)
Evaluation of herbicides for possible mutagenic properties
J. Agric. Food Chem. 20, 649-656

Anon (1988)
Pentachlorophenol
Rev. Environ. Contam. Toxicol. 104, 183-194

Ballhorn, L. et al. (1981)
Cholestyramide enhances fecal elimination of pentachlorophenol in rhesus monkeys
Chemosphere 10, 877-888

Banerjee, S. (1987)
Interrelationship between biodegradability, toxicity and structure of chlorophenols
In: K.L.E. Kaiser (Ed.) QSAR in Environmental Toxicology-II, 17-23
D. Reidel Publishing Company, Dordrecht, 1987

Bauchinger, M et al. (1982)
Chromosome damage in lymphocytes after occupational exposure to pentachlorophenol (PCP)
Mut. Res. 102, 83-88

Beelen, P. van, et al. (1989)
The influence of toxic compounds on subsoil bacteria (abstract P-9-3)
Fifth International Symposium on Microbial Ecology, 1989.8.27

Bishop, C.M. and A.H. Jones (1981)
Non-Hodgkin's lymphoma of the scalp in workers exposed to dioxins
The Lancet, 1981-II, 369

Blackburn, K. et al. (1986)
Evaluation of the reproductive toxicology of 2,4,6-trichlorophenol in male and female rats
Fundam. Appl. Toxicol. 6, 233-239

Borthwick, P.W. and S.C. Schimmel (1978)
Toxicity of pentachlorphenol and related compounds to early life stages of selected estuarine animals
In: Rao (1978), 141-146

Borzelleca, J.F. et al. (1985a)
Acute toxicity of monochlorophenols, dichlorophenols and pentachlorophenol in the mouse
Toxicol. Lett. 29, 39-42

Borzelleca, J.F. et al. (1985b)
Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice
Fundam. Appl. Toxicol. 5, 478-486

Borzelleca, J.F. et al. (1985c)
Toxicological evaluation of selected chlorinated phenols
In: R.L. Jolley et al. (Ed.), Water Chlorination: Environmental Impact and Health Effects, Volume 5, 331-343 (Chapter 26)
Lewis Publishers Inc., Chelsea

Brandt, M. et al. (1977)
Chronische Lebererkrankung durch langjährige Intoxikation im Haushalt mit Pentachlorphenol
Verh. Dtsch. Ges. Inn. Med. 83, 1609-1611

Braun, W.H. and M.W. Sauerhoff (1976)
The pharmacokinetic profile of pentachlorophenol in monkeys
Toxicol. Appl. Pharmacol. 38, 525-533

Braun, W.H. et al. (1977)
The pharmacokinetics and metabolisme of pentachlorophenol in rats
Toxicol. Appl. Pharmacol. 41, 395-406

Braun, W.H. et al. (1979)
The metabolism/pharmacokinetics of pentachlorophenol in man, and a comparison with the rat and monkey
In: Developments in Toxicology and Environmental Science, Volume 4: Toxicology and Occupational Medicine (W.B. Deichman, Ed.), 289-296, Elsevier Science Pub., Amsterdam (1979)

Buccafusco, R.J. et al. (1981)
Acute toxicity of priority pollutants to bluegill *(Lepomis macrochirus)*
Bull. Environm. Contam. Toxicol. 26, 446-452

Buselmaier, W. et al. (1972)
Mutagenitäts-untersuchungen mit Pestiziden im Host-mediated Assay und mit den Dominanten Letaltest an der Maus
Biol. Zbl. 91, 311-325

Canton, J.H. and C.J. Prins (1980)
"Milieudatasheets" van een Aantal Chloorfenolen
(Literatuursamenvatting)
RIV Rapport nr. 1/80 Doc, Rijks Instituut voor de Volksgezondheid, Bilthoven (National Institute of Public Health and Environmental Protection, The Netherlands)

Cardwell, R.D. et al. (1976)
Acute Toxicity of Selected Toxicants to Six Species of Fish
U.S. EPA Report No. 600/3-76-008, United States Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Duluth, Minnesote 55804

Casterline, J.L. et al. (1985)
Uptake, translocation and transformation of pentachlorophenol in soybean and spinach plants
Environ. Res. 37, 101-118

Chapman, G.A. and D.L. Shumway (1978)
Effects of sodium pentachlorophenate on survival and energy metabolism of embryonic and larval steelhead trout
In: Rao, K.R. (Ed.) Pentachlorophenol - Chemistry, Pharmacology and Environmental Toxicology, 285-299
Plenum, New York, 1978

Chapman, P.M. et al. (1982)
Relative tolerance of selected aquatic oligochaetes to combinations of pollutants and environmental factors
Aquat. Toxicol. 2, 69-78

Cleveland, L. et al. (1982)
Toxicity of three preparations of pentachlorophenol to fathead minnows - A comparative study
Environ. Toxicol. Chem. 1, 205-212

Conklin, P.J. and K.R. Rao (1978)
Toxicity of sodium pentachlorophenate to the grass shrimp, *Palaemonetes pugio*, in relation to the molt cycle
In: Rao (1978), 181-192

Courtney, K.D. et al., (1976)
The effects of pentachloronitrobenzene, hexachlorobenzene, and related compounds on fetal development.
Toxicol. Appl. Pharmacol. 35, 239-256

Crandall, C.A. and C.J. Goodnight (1962)
Effects of sublethal concentrations of several toxicants on growth of the common guppy, *Lebistes reticularis*
Limnol. Oceanogr. 7, 233-239

Debets, F.M. et al. (1980)
Effects of pentachlorophenol on rat liver changes induced by hexachlorobenzene, with special reference to porphyria, and alterations in mixed function oxygenases
Toxicology 15, 181-195

Denneman, C.A.J. and C.A.M. van Gestel (1990)
Bodemverontreiniging en Bodemecosystemen: Voorstel voor C-(Toetsings) Waarden op Basis van Ecotoxicologische Risico's
RIVM-rapport 725201001 (2 delen)
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Devillers, J. and P. Chambon (1986)
Acute toxicity and QSAR of chlorophenols on Daphnia magna
Bull. Environ. Contam. Toxicol. 37, 599-605

Dominguez, S.E. and G.A. Chapman (1984)
Effect of pentachlorophenol on the growth and mortality of embryonic and juvenile steelhead trout
Arch. Environ. Contam. Toxicol. 13, 739-743

Ennever, F.K. and H.S. Rosenkranz (1989)
Application of the carcinogenicity prediction and battery selection method to recent National Toxicology Program short-term test data
Environ. Molec. Mutagen. 13, 332-338

EPA (1984)
Estimating "Concern Levels" for Concentrations of Chemical Substances in the Environment
Environmental Effects Branch, Health and Environmental Review Division, Environmental Protection Agency

Ernst, W. and K. Weber (1978)
Chlorinated phenols in selected estuarine bottom fauna
Chemosphere 11, 867-872

Ernst, W. (1979)
Factors affecting the evaluation of chemicals in laboratory experiments using marine organisms
Ecotox. Environ. Saf. 3, 90-98

Exon, J.H. and L.D. Koller (1982)
Effects of transplacental exposure to chlorinated phenols
Environ. Health Perspect. 46, 137-140

Exon, J.H. and L.D. Koller (1983a)
Effects of chlorinated phenols on immunity in rats
Int. J. Immunopharmac. 5, 131-136

Exon, J.H. and L.D. Koller (1983b)
Alteration of transplacental carcinogenesis by chlorinated phenols
In: Jolley, R.L. et al. (Eds.) Water Chlorination, Environmental Impact and Health Effects, Volume 4, Chapter 84, 1177-1188, Ann Arbor Science, Mich., 1983

Exon, J.H. (1984)
A review of chlorinated phenols
Vet. Hum. Toxicol. 26, 508-520

Exon, J.H. et al. (1984)
Toxicologic, pathologic, and immunotoxic effects of 2,4-dichlorophenol in rats
J. Toxicol. Environ. Health 14, 723-730

Exon, J.H. (1985)
Bioassay of chlorinated phenolic compounds: toxicity, pathogenicity, carcinogenicity and immune modulation in rats (Dissertation)
Diss. Abst. Int. Pt. B - Sci. & Eng. 46

Exon, J.H. and L.D. Koller (1985)
Toxicity of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol
In: Jolley, R.L. et al. (eds.) Water Chlorination, Environmental Impact and Health Effects, Volume 5, Chapter 25, 307-330
Lewis Publishers Inc., Chelsea, MI, 1985

Fahrig, R. (1974)
Comparative mutagenicity studies with pesticides
In: R. Montesano and L. Tomasis (Eds), Chemical Carcinogenesis Essays
IARC Scientific Publications 10, 161-181

Fahrig, R. et al. (1978)
Genetic activity of chlorophenols and chlorophenol impurities
In: Rao, K.R. (1978), 325-338

Firestone, D. et al. (1979)
Polychlorodibenzo-p-dioxin and pentachlorophenol residues in milk and blood of cows fed technical pentachlorophenol
J. Agric. Food Chem. 27, 1171-1177

Fiskesjö, G. et al. (1981)
Chlorinated phenoxyacetic acids and chlorophenols in the modified Allium test
Chem. Biol. Interact. 34, 333-344

Fogels, A. and J.B. Sprague (1977)
Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants
Water. Res. 11, 811-817

Freeman, L. (1953)
A standardized method for determining toxicity of pure compounds to fish
Sewage and Industrial Wastes 25, 845-848

Galloway, S.M. et al. (1987)
Chromosome aberrations and sister chromatid exchange tests in vitro in Chinese hamster ovary cells: Results for 108 chemicals
Environ. Molec. Mutagen. 10, Suppl. 10, 1-75

Gersich, F.M. and D.P. Millazzo (1990)
Evaluation of a 14-day static renewal toxicity test with *Daphnia magna* Straus
Arch. Environ. Contam. Toxicol. 19, 72-76

Gestel, C.A.M. van, et al. (1987)
Toxiciteit en Bioaccumulatie van Chloorfenolen in Regenwormen in Relatie tot de Beschikbaarheid in de Bodem
RIVM-rapport nr. 718479001 / RIN-rapport nr. 87/12
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Gestel, C.A.M. van, and Wei-chun Ma (1988)
Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil
Ecotox. Environ. Saf. 15, 289-297

Gestel, C.A.M. van, and W.A. Van Dis (1988)
The influence of soil characteristics on the toxicity of four chemicals to the earthworm *Eisenia fetida andrei* (Oligochaeta)
Biol. Fertil. Soils 6, 262-265

Gestel, C.A.M. van, et al. (1988)
Development of a standardized reproduction test with the earthworm species *Eisenis fetida andrei* using copper, pentachlorophenol, and 2,4-dichloroaniline.
Paper presented on the 1st European Conference on Ecotoxicology. 17-19 October, 1988, Copenhagen, Denmark
Exotox. Environ. Saf. 18, 305-312 (1989)

Gestel, C.A.M. van, et al. (1990)
An approach to quantitative structure-activity relationships (QSARs) in terrestrial ecotoxicology: earthworm toxicity studies
Submitted to "Chemosphere"

Gezondheidsraad (1988)
Ecotoxicologische Risico-evaluatie van Stoffen
Rapport 1988/28, 's-Gravenhage, The Netherlands
(An English translation of this Health Council report: "Analyzing the Risk of Toxic Chemicals for Ecosystems" is available)

Goldstein, J.A. et al. (1977)
Effects of pentachlorophenol on hepatic drug-metabolizing enzymes and porphyria related to contamination with chlorinated dibenzo-p-dioxins and dibenzofurans
Biochem. Pharmacol. 26, 1549-1557

Greene, M.H. (1978)
Familial and sporadic Hodgkin's disease associated with occupational wood exposure
The Lancet, 1978-II, 626-627

Greichus, Y.A. et al. (1979)
Diagnosis and physiologic effects of pentachlorophenols on young pigs. Part I. Effects of purified pentachlorophenol
Bull. Environm. Contam. Toxicol. 23, 418-422

Hamilton, S.J. et al. (1986)
Toxicity of pure pentachlorophenol and chlorinated phenoxyphenol impurities to fathead minnows
Environ. Toxicol. Chem. 5, 543-552

Hanstveit, A.O. (1980)
Evaluation of the Results of the European ISO-test Program with Algal Toxicity Tests
ISO/TC 147/SC 5/WG 5 No. 16, Nederlands Normalisatie Instituut, Delft

Haque, A. and W. Ebing (1986)
Uptake and accumulation of pentachlorophenol and sodium pentachlorophenate by earthworms from water and soil
Sci. Total Environ. 68, 113-125

Haque, A. et al. (1988)
Environmental fate and distribution of sodium [^{14}C]pentachlorophenate in a section of urban wasteland ecosystem
Sci. Total Environ. 68, 127-139

Hattula, M.L. et al. (1981a)
Acute toxicity of some chlorinated phenols, catechols and cresols to trout
Bull. Environm. Contam. Toxicol. 26, 295-298

Hattula, M.L. et al. (1981b)
Acute and short-term toxicity of 2,3,4,6-tetrachlorophenol in rats
Bull. Environm. Contam. Toxicol. 26, 795-800

Hattula, M.L. and J. Knuutinen (1985)
Mutagenesis of mammalian cells in culture by chlorophenols, chlorocatechols and chloroguaiacols
Chemosphere 14, 1617-1625

Haworth, S. et al. (1983)
Salmonella mutagenicity test results for 250 chemicals
Environ. Mutagen. Suppl. 1, 3-142

Hedtke, S.F. et al. (1986)
Toxicity of pentachlorophenol to aquatic organisms under naturally varying and controlled environmental conditions
Environ. Tox. Chem. 5, 531-542

Heitmuller, P.T. et al. (1981)
Acute toxicity of 54 industrial chemicals to sheepshead minnows *(Cyprinodon variegatus)*
Bull. Environm. Contam. Toxicol. 27, 596-604

Hillam, R.P. (1983)
Effects of purified pentachlorophenol on the serum proteins of young pigs
Bull. Environ. Contam. Toxicol. 31, 599-604

Hinkle, D.K. (1973, abstract)
Fetotoxic effects of pentachlorophenol in the golden Syrian hamster
Abstracts of Papers for the Twelfth Annual Meeting of the Society of Toxicology, New York, March 18-22, 1973
Toxicol. Appl. Pharmacol. 25, 455

Hoben, H.J. et al. (1976a)
A study of inhalation of pentachlorophenol by rats, III. Inhalation toxicity study
Bull. Environ. Contam. Toxicol. 15, 463-465

Hoben, H.J. et al. (1976b)
A study of inhalation of pentachlorophenol by rats, IV. Distribution and excretion of inhaled pentachlorophenol
Bull. Environ. Contam. Toxicol. 15, 466-474

Hodson, P.V. & B.R. Blunt (1981)
Temperature-induced changes in pentachlorophenol chronic toxicity to early life stages of rainbow trout
Aquat. Toxicol. 1, 113-127

Holcombe, G.W. et al. (1982)
Effects of phenol, 2,4-dimethylphenol, 2,4-dichlorophenol, and pentachlorophenol on embryo, larval, and early-juvenile fathead minnows *(Pimephales promelas)*
Arch. Environ. Contam. Toxicol. 11, 73-78

Holcombe, G.W. et al. (1984)
The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to fathead minnows *Pimephales promelas*
Environ. Pollut. (Series A) 35, 367-381

Holzapple, M.P. et al. (1987)
Effects of pentachlorophenol on the *in vitro* and *in vivo* antibody response
J. Toxicol. Environ. Health 20, 229-239

Hooftman, R.N. and G.J. Vink (1980)
The determination of toxic effects of pollutants with the marine polychaete worm *Ophryotrocha diadema*
Ecotoxicol. Environ. Saf. 4, 252-262

Huang, J-C. and E.F. Gloyna (1968)
Effect of organic compounds on photosynthetic oxygenation - I. Chlorophyll destruction and suppression of photosynthetic oxygen production
Water Res. 2, 347-366

Hughes, B.J. et al. (1985)
Assessment of pentachlorophenol toxicity in newborn calves: clinicopathology and tissue residues
J. Animal. Sci. 61, 1587-1603

Hulzebos, E.M. et al. (1989)
Toxiciteit van 45 Prioritaire Organische Stoffen voor Sla *(Lactuca sativa)*, RIVM-rapport 718710002, Eindrapport van het RIVM-aandeel in het Project Fytotoxiciteit 2
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Ingols, R.S. et al. (1966)
Biological activity of halophenols
Journal WPCF 38, 629-635

Innes, J.R.M. et al. (1969)
Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note
J. Nat. Cancer Inst. 42, 1101-1114

Janssen, J.J. and P.J.C. Schepens (1985)
Chronic poisoning by wood preservatives in houses
Belg. Arch. Soc. Gen., Hyg., Arbeidsg. en Ger. Gen. 43, 536-550

Jansson, K. and V. Jansson (1986)
Inability of chlorophenols to induce 6-thioguanine-resistant mutants in V79 Chinese hamster cells
Mut. Res. 171, 165-168

Johnson, R.L. et al. (1973)
Chlorinated dibenzodioxins and pentachlorophenol
Environ. Health Perspect. 5, 171-175

Kaila, K. and J. Saarikoski (1977)
Toxicity of pentachlorophenol and 2,3,6-trichlorophenol to the crayfish *(Astacus fluviatilis* L.)
Environ. Pollut. 12, 119-123

Kaiser, K.L.E. et al. (1984)
QSAR studies on chlorophenols, chlorobenzenes and para-substituted phenols
In: K.L.E. Kaiser (Ed.) (1984), 189-206

Kaiser, K.L.E. (Ed.)(1984)
QSAR in Environmental Toxicology.
Proceedings of the Workshop on Quantitative Structure-activity Ralationships (QSAR) in Environmental Toxicology held at McMaster University, Hamilton, Ontario, Canada, August 16-18, 1983
D. Reidel Publishing Company, Dordrecht, 1984

Kappers, F.I. and J.A.A.M. Wondergem-Van Eijk (1989)
Effecten van chloorfenolen op vrijlevende bodemnematoden
Rapportnr. 718602003, Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven (National Institute of Public Health and Environmental Protection, The Netherlands)

Kerkvliet, N. I. et al. (1982)
Immunotoxicity of pentachlorophenol (PCP): increased susceptibility to tumor growth in adult mice fed technical PCP-contaminated diets
Toxicol. Appl. Pharmacol. 62, 55-64

Kessler, F.K. et al.
Effects of chlorinated phenols on the mouse bone marrow sister chromatid exchange (abstract)
Published abstract; source unknown.

Kimbrough, R.D. and R.E. Linder (1978)
The effect of technical and purified pentachlorophenol on the rat liver
Toxicol. Appl. Pharmacol. 46, 151-162

Kinzell, J.H. et al. (1981)
Subchronic administration of technical pentachlorophenol to lactating dairy cattle: performance, general health, and pathologic changes
J. Dairy Sci. 64, 42-51

Kinzell, J.H. et al. (1985)
Metabolic fate of [U-^{14}C]pentachlorophenol in a lactating cow
J. Agric. Food Chem. 33, 827-833

Klemmer, H.W. et al. (1980)
Clinical findings in workers exposed to pentachlorophenol
Arch. environm. Contam. toxicol. 9, 715-725

Knudsen, I. et al. (1974)
Short-term toxicity of pentachlorophenol in rats
Toxicology 2, 141-152

Kobayashi, S. et al. (1972)
Chronic toxicity of 2,4-DCP in mice: a simnple design for the toxicity of residual metabolites of pesticides (Partly in Japanese).
J. Med. Soc. Toho Japan 19, 356-362

Kobayashi, K. et al. (1979)
Relationship between toxicity and accumulation of various chlorophenols in goldfish
Bull. Japan. Soc. Sci. Fish. 45, 173-175

Kobayashi, K. and T. Kishino (1980)
Effect of pH on the toxicity and bioaccumulation of pentachlorophenol in goldfish
Bull. Japan. Soc. Sci. Fish. 46, 167-170

Kolosova, L and N. Stroganov
Analysis of the mechanism of some pesticides on *Daphnia* according to biological indices (In Russian)
Eksp. Vodn. Toksikol 5, 134-145

Könemann, H. (1979)
Quantitative structure-activity relationships for kinetics and toxicity of aquatic pollutants and their mixtures in fish
Thesis State University Utrecht, The Netherlands
Chapter 4: Quantitative structure-activity relationships in fish toxicity studies, Part 2: The influence of pH on the QSAR of chlorophenols (also published in "Toxicology", vol 19, 223-228, Könemann, H. and A. Musch)
Chapter 5: Fish toxicity tests with mixtures of more than two chemicals: A proposal for a quantitative approach and experimental results (also published in "Toxicology", vol. 19, 229-238)

Kooijman, S.A.L.M. (1987)
A safety factor for LC50-values allowing for differences in sensitivity among species
Wat. Res. 21, 269-276

Kopperman, H.L. et al. (1974)
Aqueous chlorination and ozonation studies I. Structure-toxicity correlations of phenolic compounds to *Daphnia magna*
Chem.-Biol. Interactions 9, 245-251

Koss, G. and W. Koransky (1978)
Pentachlorophenol in different species of vertebrates after administration of hexachlorobenzene and pentachlorobenzene
In: Rao, K.R. (1978), 131-...

Krause, CHr. and N. Englert (1980)
Health evaluation of pentachlorophenol containing wood preservatives in rooms (In German)
Holz Roh Werkst. 38, 429-432

Krijgsheld, K.R. and A. van der Gen (1986)
Assessment of the impact of the emissions of certain organochlorine compounds on the aquatic environment. Part 1. Monochlorophenols and 2,4-dichlorophenol
Chemosphere 15, 825-860

Kühn, R. et al. (1989a)
Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*
Wat. Res. 23, 495-499

Kühn, R. et al. (1989b)
Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test
Wat. Res. 23, 501-510

Larsen, R.V. et al. (1975)
Placental transfer and teratology of pentachlorophenol in rats
Envir. Lett. 10, 121-128

LeBlanc, G.A. (1980)
Acute toxicity of priority pollutants to water flea *(Daphnia magna)*
Bull. Environm. Contam. Toxicol. 24, 684-691

LeBlanc, G.A. (1984a)
Interspecies relationships in acute toxicity of chemicals to aquatic organisms
Environ. Toxicol. Chem. 3, 47-60

LeBlanc, G.A. (1984b)
Comparative structure-activity relationships between acute and chronic effects to aquatic organisms
In: K.L.E. Kaiser (1984).

LeBlanc, G.A. et al. (1988)
Relationships between the structures of chlorinated phenols, their toxicity, and their ability to induce glutathione S-transferase activity in *Daphnia magna*
Aquat. Toxicol. 12, 147-156

Leeuwen. C.J. van, et al. (1985)
Differences in susceptibility of early life stages of rainbow trout *(Salmo gairdneri)* to environmental pollutants
Aquat. toxicol. 7, 59-78

Leeuwen, C.J. van, et al. (1987)
Effects of chemical stress on the population dynamics of *Daphnia magna:* a comparison of two test procedures
Ecotox. Environ. Saf. 14, 1-11

Lilienblum, W. (1985)
Formation of pentachlorophenol glucuronide in rat and human liver microsomes
Biochem. Pharmacol. 34, 893-894

Liu, D. et al. (1982)
Quantitative structure-toxicity relationship of halogenated phenols on bacteria
Bull. Environm. Contam. Toxicol. 29, 130-136

Matida, Y. et al. (1970)
Study on the toxicity of agricultural control chemicals in relation to freshwater fisheries management No. 5. Some effects of sodium pentachlorophenate to freshwater fishes
Bull. Freshwater Fish. Res. Lab. 20, 127-145

Mayer, F. L., Jr. and M.R. Ellersieck (1986)
Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals
Unites States Department of the Interior, Fish and Wildlife Service, Resource Publication 160, Washington D.C.

Mayes, M.A. et al. (1983)
A study to assess the influence of age on the response of fathead minnows in static acute toxicity tests
Bull. Environ. Contam. Toxicol. 31, 139-147

McCollister, D.D. et al. (1961)
Toxicologic information on 2,4,5-trichlorophenol
Toxicol. Appl. Pharmacol. 3, 63-70

McConnell, E.E. et al. (1980)
The chronic toxicity of technical and analytical pentachlorophenol in cattle. I. Clinicopathology
Toxicol. Appl. Pharmacol. 52, 468-490

McGregor, D.B. et al. (1988)
Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation: III. 72 coded chemicals
Environ. Molecul. Mutagen 12, 85-154

Meent, D. van de, et al. (1990)
Streven naar Waarden. Achtergrondstudie ten behoeve van de Nota "Milieukwaliteitsnormering Water en Bodem"
RIVM-rapport 670101001 (2 delen)
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Moriya, M. et al. (1983)
Further mutagenicity studies on pesticides in bacterial reversion assay systems
Mut. Res. 116, 185-216

Mount, D.I. and T.J. Norberg (1984)
A seven-day life-cycle cladoceran toxicity test
Environ. Toxicol. Chem. 3, 425-434

Nagler, J.J. et al. (1986)
Effect of sublethal pentachlorophenol on early oogenesis in maturing female rainbow trout *(Salmo gairdneri)*
Arch. Environ. Contam. Toxicol. 15, 549-555

NCI (1979)
Bioassay of 2,4,6-T3CP for Possible Carcinogenicity
NCI-CG-TR-155, National Cancer Institute, Technical Report Series No. 155, U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda
DHEW Publication No. (NIH) 79-1711

Nestmann, E.R. et al. (1980)
Mutagenicity of constituents identified in pulp and paper mill effluents using the *Salmonella* /mammalian-microsome assay
Mut. Res. 79, 203-212

Nestmann, E.R. and E.G.-H. Lee (1983)
Mutagenicity of constituents of pulp and paper mill effluents in growing cells of Saccharomyces cerevisiae
Mut. Res. 119, 273-280

Neuhauser, E.F. et al. (1986)
Comparative toxicity of ten organic chemicals to four earthworm species
Comp. Biochem. Physiol. 83C, 197-200

Niimi, A.J. and C.A. McFadden (1982)
Uptake of sodium pentachlorophenate (NaPCP) from water by rainbow trout *(Salmo gairdneri)* exposed to concentrations in the ng/l range
Bull. Environm, Contam. Toxicol. 28, 11-19

Nishimura, N. et al. (1982)
Survey on mutagenicity of pesticides by the *salmonella* -microsome test
J. Aichi. Med. Univ. Assoc. (Aichi Ika Daigaku Igakukai Zasshi) 10, 305-312

Norberg-King, T.J (1989)
An evaluation of the fathead minnow seven-day subchronic test for estimating chronic toxicity
Environ. Toxicol. Chem. 8, 1075-1089

NRCC (1982)
Chlorinated Phenols: Criteria for Environmental Quality
NRCC No. 18578, Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Ottawa

NTP (1989a)
Toxicology and Carcinogenesis Studies of 2,4-Dichlorophenol (CAS No. 120-83-2) in F344/N Rats and B6C3F1 Mice (Feed Studies)
National Toxicology Program Technical Report Series No. 353, June 1989; U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH Publication No. 89-2808)

NTP (1989b)
Toxicology and Carcinogenesis Studies of Two Pentachlorophenol Technical-grade Mixtures (CAS No. 87-86-5) in B6C3F1 Mice (Feed Studies)
National Toxicology Program Technical Report Series No. 349, March 1989; U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH Publication No. 89-2804

Okkerman, P.C. et al. (1990)
Validatie van een Aantal Extrapolatiemethoden met Toxiciteitsgegevens afkomstig uit Multiple Species-Experimenten
RIVM-rapport 670206001
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Olivier, L. and W.T. Haskins (1960)
The effects of low concentrations of sodium pentachlorophenate on the fecundity and egg viability of *Australorbis glabratus*
Am. J. Trop. Med. Hyg. 9, 199-205

Osterloh, J. et al. (1983)
An assessment of the potential testicular toxicity of 10 pesticides using the mouse-sperm morphology assay
Mut. Res. 116, 407-415

Parrish, P.R. et al. (1978)
Chronic toxicity of chlordane, trifluralin, and pentachlorophenol to sheepshead minnows *(Cyprinodon variegatus)*
Report No. EPA-600/3-78-010, United States Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Gulf Breeze, Florida 32561

Phipps, G.L. et al. (1981)
The acute toxicity of phenol and substituted phenols to the fathead minnow
Bull. Environ. Contam. toxicol. 26, 585-593

Posthuma, R. (1988)
Toxiciteit van pentachloorfenol en 2,4-dichlooraniline voor regenwormen en slaplanten (Studentenverslag)
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven

Prescott, C.A. et al. (1982)
Influence of a purified grade of pentachlorophenol on the immune response of chickens
Am. J. Vet. Res. 43, 481-487

Probst, G.S. et al. (1981)
Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds
Environ. Mutagen. 3, 11-32

Ramel, C. and J. Magnussen (1979)
Chemical induction of nondisjunction in Drosophila
Environ. Health Perspect. 31, 59-66

Rao, K.R. (Ed) (1978)
Pentachlorophenol: Chemistry, Pharmacology, and Environmental Toxicology
International symposium on Pentachlorophenol, Pensacola, Florida, June 27-29, 1977
Plenum Press, New York, London

Rapson, W.H. et al. (1980)
Mutagenicity produced by aqueous chlorination of organic compounds
Bull. Environ. Contam. Toxicol. 24, 590-596

Rasanen, L. et al. (1977)
The mutagenicity of MCPA and its soil metabolites, chlorinated phenols, catechols and some widely used slimicides in Finland
Bull. Environ. Contam. Toxicol. 18, 565-571

Roberts, B.L. and H.W. Dorough (1984)
Relative toxicities of chemicals to the earthworm *Eisenia foetida*
Environ. Toxicol. Chem. 3, 67-78

Rodwell, D.E. et al. (1989)
Teratogenic assessment of 2,4-dichlorophenol in Fischer 344 rats
Fundam. Appl. Toxicol. 13, 635-640

RTECS (1989, on-line data base)
Registry of Toxic effects of Chemical substances
U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health

Rubinstein, N.I. (1978)
Effect of sodium pentachlorophenate on the feeding activity of the lugworm, *Arenicola cristata* Stimpson
In: Rao (1978), 175-179

Rübelt, C. et al. (1982)
Schadstoffe im Wasser - Band II. Phenole
Deutsche Forschungsgemeinschaft, Harald Boldt Verlag, Boppard

Saarikoski, J. and M. Viluksela (1981)
Influence of pH on the toxicity of substituted phenols to fish
Arch. Environm. Contam. Toxicol. 10, 747-753

Saarikoski, J. and M. Viluksela (1982)
Relationship between physicochemical properties of phenols and their toxicity and accumulation in fish
Ecotoxicol. Environ. Saf. 6, 501-512

Sangster, B et al. (1982)
Non-occupational exposure to pentachlorophenol: clinical findings and plasma-PCP-concentrations in three families
Human Toxicol. 1, 123-133

Schimmel, S.C. et al. (1978)
Effects of sodium pentachlorophenate on several estuarine animals: toxicity, uptake and depuration
In Rao (1978), 147-155

Schultz, T. W. et al. (1986)
Relationships of quantitative structure-activity to comparative toxicity of selected phenols in the *Pimephales promelas* and *Tetrahymena pyriformis* test systems
Ecotoxicol. Environ. Saf. 12, 146-153

Schultz, T.W. (1987)
The use of the ionization constant (pK_a) in selecting models of toxicity in phenols
Ecotoxicol. Environ. Saf. 14, 178-183

Schwetz, B.A. et al. (1974a)
Effects of purified and commercial grade tetrachlorophenol on rat embryonal and fetal development
Toxicol. Appl. Pharmacol. 28, 146-150

Schwetz, B.A. et al. (1974b)
The effect of purified and commercial grade pentachlorophenol on rat embryonal and fetal development
Toxicol. Appl. Pharmacol. 28, 151-161

Schwetz, B.A. et al. (1978)
Results of two-year toxicity and reproduction studies on pentachlorophenol in rats
In: Rao, K.R. (1978), pp 301-309

Seyler, D.E. et al. (1984)
The use of *in vitro* methods for assessing reproductive toxicity. Dichlorophenols
Toxicol. Lett. 20, 309-315

Shen, S.Y. et al. (1983)
Acute dermal toxicity of tetrachlorophenols in the rat
Bull. Environ. Contam. Toxicol. 31, 680-685

Shigeoka, T. et al. (1988a)
Acute toxicity of chlorophenols to green algae, *Selenastrum capricornutum* and *Chlorella vulgaris*, and quantitative structure-activity relationships
Environ. Toxicol. Chem. 7, 847-854

Shigeoka, T. et al. (1988b)
Toxicity and QSAR of chlorophenols on Daphnia
EISEI KAGAKU 34, 169-175

Shirasu, Y. (1975)
Significance of mutagenicity testing on pesticides
Environ. Qual. Saf. 4, 226-231

Simmon, V.F. et al. (1977)
Mutagenic activity of chemicals identified in drinking water
Dev. Toxicol. Environ. Sci. 2, 249-258

Slooff, W. and J.H. Canton (1983)
Comparison of the susceptibility of 11 freshwater species to 8 chemical compounds. II. (Semi)chronic toxicity tests
Aquat. Toxicol. 4, 271-282

Slooff, W. et al. (1983)
Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. (Sub)acute toxicity tests
Aquat. Toxicol. 4, 113-128

Slooff, W. et al. (1986)
Margins of uncertainty in ecotoxicological hazard assessment
Environ. Toxicol. Chem. 5, 841-852

Slooff et al. (1990)
Basisdocument Chloorfenolen (Integrated Criteria Document Chlorophenols)
Rapport nr. 710401003, Rijksinstituut voor Volksgezondheid en Milieuhygiëne (National Institute of Public Health and Environmental Protection), Bilthoven, the Netherlands
(An English translation of the criteria document will be available in 1991)

Snell, T.W. and G. Persoone (1989)
Acute toxicity bioassays using rotifers. I. A test for brackish and marine environments with Brachionus plicatilis
Aquat. Toxicol. 14, 65-80

Spehar, R.L. et al. (1985)
Pentachlorophenol toxicity to amphipods ansd fathead minnows at different pH values
Environ. Toxicol. Chem. 4, 389-397

Stedman, T.M. Jr. et al. (1980)
Toxicity and bioaccumulation of pentachlorophenol in broiler chickens
Poult. Sci. 59, 1018-1026

Sterling, T.D. et al. (1982)
Health effects of chlorophenol wood preservatives on sawmill workers
Int. J. Health Services 12, 559-571

Straalen, N.M. van, and C.A.J. Denneman (1989)
Ecotoxicological evaluation of soil quality criteria
Ecotoxicol. Environ. Saf. 18, 241-251

Strobel, K. and T. Grummt (1987)
Aliphatic and aromatic halocarbons as potential mutagens in drinking water
Toxicol. Environ. Chem. 14, 143-156

Tam, T.-Y. and J.T. Trevors (1981)
Effects of pentachlorophenol on asymbiotic nitrogen fixation in soil
Water, Air, Soil Pollut. 16, 409-414

Uhl, S. et al. (1986)
Pharmacokinetics of pentachlorophenol in man
Arch. Toxicol. 58, 182-186

Vogel, E. and J.L.R. Chandler (1974)
Mutagenicity testing of cyclamate and some pesticides in drosophila melanogaster
Experientia 30, 621-623

Welsh, J.J. et al. (1987)
Teratogenic potential of purified pentachlorophenol and pentachloroanisole in subchronically exposed Sprague-Dawley rats
Food Chem. Toxicol. 25, 163-172

WHO (1987)
Environmental Health Criteria 71: Pentachlorophenol
IPCS International Programme on Chemical Safety,
World Health Organization, Geneva

WHO (1989)
Environmental Health Criteria 93: Chlorophenols other than PCP
IPCS International Programme on Chemical Safety,
World Health Organization, Geneva

Witte, I. et al. (1985)
DNA-damaging properties and cytotoxicity in human fibroblasts of tetrachlorohydroquinone, a pentachlorophenol metabolite
Mut. Res. 145, 71-85

Wyllie, J.A. et al. (1975)
Exposure and contamination of the air and employees of a pentachlorophenol plant, Idaho-1972
Pestic. Monit. J. 9, 150-152

Zeiger, E. et al. (1988)
Salmonella mutagenicity tests. IV. Results from the testing of 300 chemicals
Environ. Molec. Mutagen. 11 (Suppl. 12), 1-158

Zelles, L. et al. (1986)
Comparison of methods to test chemicals for side effects on soil microorganisms
Ecotoxicol. Environ. Saf. 12, 53-69

Ziemsen, B. et al. (1987)
Sister chromatid exchange and chromosomal breakage in pentachlorophenol (PCP) exposed workers
Int. Arch. Occup. Environ. Health 59, 413-417

Zomer, G. (1990)
QSAR for Chlorophenols
RIVM-rapport 32900103 (in preparation)
Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven