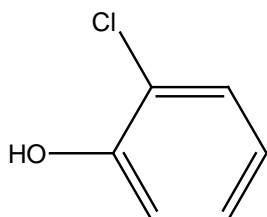


COMMITTEE ON THE MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT**REVIEW OF GENOTOXICITY OF CHLOROPHENOLS****Referral to COM**

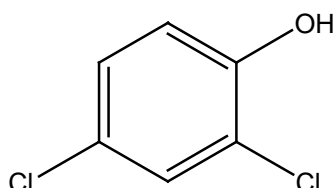
1. The Food Standards Agency has asked for advice on the genotoxicity of chlorophenol compounds to assist in developing advice on their occurrence as contaminants in wine.

Introduction

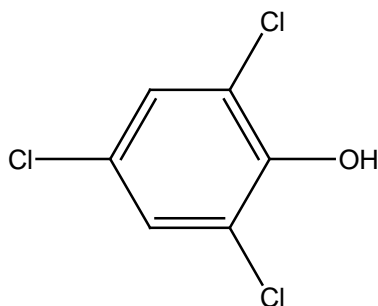
2. Chlorophenols are organic chemicals formed from phenol (1-hydroxybenzene) by substitution in the phenol ring with one or more atoms of chlorine. Nineteen congeners are possible, ranging from monochlorophenols to the fully chlorinated pentachlorophenol. Chlorophenols, particularly trichlorophenols, tetrachlorophenols, and pentachlorophenol, are also available as sodium or potassium salts. Examples of chlorophenol structures are given below:



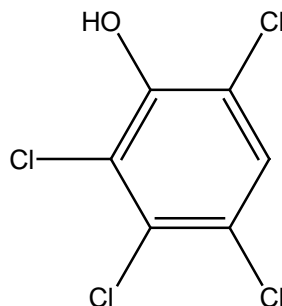
2-chlorophenol



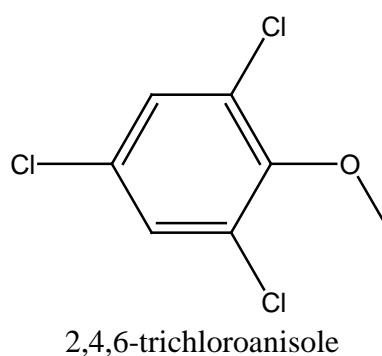
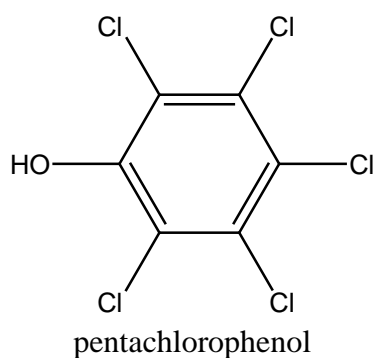
2,4-dichlorophenol



2,4,6-trichlorophenol



2,4,5,6-tetrachlorophenol



3. Chlorophenols are toxic to a wide range of organisms, a property that accounts for many of their uses. Higher chlorophenols (CPs) have been used in the wood preservation industry; for example, substantial amounts of the sodium salts of tetrachlorophenol (TeCP), pentachlorophenol (PCP), and trichlorophenol (TCP) were used to surface-treat fresh-cut logs and lumber against sapstain fungi and surface mould. Lower chlorophenols served as intermediates in the production of pesticides, such as TCP, PCP, 2,4-dichlorophenol (2,4-DCP), and 2,4,5-trichlorophenol (2,4,5-TCP). The use of 2,4,5-TCP has been discontinued in a number of countries. Lesser amounts of chlorophenols have been used as wood preservatives in agricultural and domestic applications, and as additives to inhibit microbial growth in a wide array of products, such as adhesives, oils, textiles, and pharmaceutical products. Industrially produced chlorophenols may be contaminated with compounds such as polychlorinated dibenzodioxins and polychlorinated dibenzofurans (IARC, 1986; McConnell *et al*, 1991). As discussed in paragraph 12 below, chlorophenols are not currently permitted biocides or pesticides within the EU.
4. Most human data on the toxicity of chlorophenols come from studies in occupational environments such as the leather and wood industries as well as use within the pesticide industry. Occupational exposure to chlorophenols has been linked to increased incidences of cancer (IARC, 1986); irritation to the skin, eye nose and airways as well as dermatitis, chloracne, porphyria, abnormal liver function tests, neurological and immune effects have also been noted (IARC, 1986, 1999; WHO, 1989).
5. Low levels of chlorophenols are also found as contaminants in wine. The main source is via contamination of the cork. The cork trees used to manufacture the corks for the wine bottles can be contaminated with chlorophenols if they are deposited on soil contaminated with pesticides or wood preservatives. The

sterilisation of corks by chlorine bleaching can also result in formation of chlorophenols, which will remain on the cork if not washed off properly. However, this source is becoming less common due to increased use of peroxide instead of chlorine bleaching (Wikipedia, 2011). The chlorophenols detected include 2,4,6-TCP, 2,4-DCP and 2,6-DCP. Analysis for 2-CP, 4-CP, 2,3,4,6-Te CP and 2,3,5,6-TeCP may also be conducted. Analytical data suggest that low levels of chlorophenols may occur naturally in some wines as well as occurring through contamination (Campden BRI, personal communication). The chlorophenols can subsequently form their corresponding chloroanisoles (eg 2,4 DCP would form 2,4-dichloroanisole etc). 2,4,6-trichloroanisole (TCA) is a common cause of cork taint (Álvarez-Rodríguez *et al*, 2002), found in approximately 80% of cases. It is formed when chlorophenol compounds are metabolised by naturally occurring airborne fungi such as *Aspergillus sp.* and *Penicillium sp.* Literature searched for chloroanisole[s] + toxicity has not revealed any published data for the chloroanisoles with the exception of pentachloroanisole. This is discussed later in this paper (paragraphs 171-176).

6. Background levels of CPs are in the range 0.5-1 µg/L (Campden BRI, personal communication) and they may become organoleptically detectable at approximately 4 µg/L. At background levels of CP exposure (0.5-1 µg/L) the exposure from 21 units of average wine (the maximum recommended weekly consumption for men and assuming 1.5 units per 125 ml glass = 1.75L wine/week) would be 0.875-1.75 µg CPs or 0.015-0.029 µg/kg bw for a 60 kg adult. At levels of 4 µg/L the exposure from 21 units of average wine would be 7µg CPs or 1.16 µg/kg bw for a 60 kg adult, above this level the CPs should be organoleptically detectable less palatable, reducing consumption.
7. The Food Standards Agency has been asked to comment on the occurrence of chlorophenols in wine. Previously the FSA advice has been that it would be prudent to assume that the compounds are genotoxic and thus exposure should be as low as reasonably practicable (ALARP). It is hoped that advice from COM will allow this to be refined.

Previous assessments.

8. The chlorophenols as a group have been classified as IARC group 2B carcinogens, ie, possibly carcinogenic to humans (IARC, 1987). This classification is based on limited evidence of carcinogenicity in humans exposed during the production of chlorophenoxy herbicides and sufficient animal evidence of carcinogenicity for 2,4,6-TCP, based on an increase in hepatocellular carcinomas or adenomas in mice of each sex and an increased incidence of lymphomas and leukaemias in male rats (NCI, 1979). The

evidence of carcinogenicity in animals for 2,4,5-TCP was considered inadequate (IARC,1987). The chlorophenols were reviewed again by IARC in 1999 and remained classified as group 2B based on limited evidence of carcinogenicity of combined exposures in humans to chlorophenols or their sodium salts. There was evidence of lack of carcinogenicity for 2,4-DCP in experimental animals, inadequate evidence of carcinogenicity in experimental animals for 2,4,5-TCP, limited experimental animal evidence of carcinogenicity for 2,4,6-TCP, and sufficient evidence in experimental animals for the carcinogenicity of PCP (IARC,1999).

9. The US Agency for Toxic Substances and Disease Registry (ATSDR) review of the chlorophenols noted that exposed pesticide workers might be at increased risk of soft tissue sarcoma as reported in some, but not all, occupational studies (ATSDR, 1999). Possible confounders were noted to be recall and selection bias, but the presence of chlorinated dioxins as contaminants in chlorophenol-containing pesticides was also pointed out. The ATSDR considered that the results of the animal studies partially supported the human data with 2,4,6-TCP being a carcinogen in 2 year rat and mice studies. Other studies suggested that chlorophenols were not directly mutagenic.
10. The US EPA has classified 2,4,6-TCP as a class 2B carcinogen (EPA, 1987). This means that it is considered to be a probable human carcinogen based on no human data and sufficient animal data, that is, an increase in hepatocellular carcinomas or adenomas in mice of each sex and an increased incidence of lymphomas and leukaemias in male rats (NCI,1979).
11. The COM considered phenol in 2008 and concluded that phenol was mutagenic “*in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage” (COM, 2008).

Regulatory levels

12. There are no chlorophenols authorised for use in the UK as pesticides or biocides. In the EU, no chlorophenols are permitted as pesticide active substances. However, although no chlorophenols are authorised in the EU as biocides, PCP and 2,4,6-TCP were identified as existing substances which could continue to be authorised under traditional arrangements pending review. Therefore it is possible that these two chlorophenols may have uses in some EU member states. Although not approved in the EU as a pesticide,

PCP has a default maximum residue level (MRL) in raw agricultural products for human consumption of 0.01 mg/kg.

13. 2,4- DCP is used in the production of certain herbicides approved in the EU and has been found as an impurity and metabolite. 2,4,6-TCP is both is also known to be an impurity and metabolite of a pesticide used in the UK.
14. Limits and guidelines for chlorophenols as disinfection by-products have been set for drinking water by EPA and WHO respectively, based on organoleptic rather than toxicological criteria (WHO, 1984). There are no specific EU regulations for chlorophenols in drinking water.

Physical and Chemical Properties

15. Chlorophenols are solids at room temperature, except for 2-CP which is a liquid. They have strong odours described as pungent or medicinal, particularly those of 2-CP and 2,4-DCP.
16. Although the solubility in water of all chlorophenols is poor, varying from 2.1×10^{-1} mol/L for 2-CP to 7.9×10^{-4} mol/L for 2,3,4,6-TeCP (ATSDR, 1999) they readily dissolve in a number of organic solvents. In contrast, the sodium or potassium salts of chlorophenols are up to four orders of magnitude more soluble in water than the parent compounds. The acidity of chlorophenols increases as the number of chlorine substitutions increases. Thus, ionization of the higher chlorophenols begins at a lower pH than that of the lower chlorophenols (pH approximately 3.5 versus 7 for PCP and 2-CP, respectively), with important implications for the interactions between pH and chlorophenol sorption or toxicity. The n-octanol-water partition coefficient of chlorophenols also increases with chlorination, indicating a propensity of the higher chlorophenols to bioaccumulate (WHO, 1989). Bioaccumulation in the environment appears to be moderate and bioconcentration appears to be a function of chlorine number and unrelated to the particular organisms. Once exposure is ceased the CPs are readily cleared from biota (WHO, 1989). This is supported by the observed increase in the clearance time for chlorophenols in laboratory animals with increasing chlorination.

Kinetics and Metabolism

Absorption

17. The majority (80-90%) of orally-administered TCP and TeCP is recovered in the urine of test animals, with much lower amounts being recovered in the

faeces indicating that they are readily absorbed through the gastrointestinal tract. Similarly, more than 90% of an oral dose of PCP was excreted in the urine of volunteers, indicating similarly effective absorption via the gastrointestinal tract in humans (Braun *et al.*, 1979).

Distribution

18. Once absorbed, the chlorophenols are widely distributed through the body and do not appear to be preferentially accumulated or stored by any particular tissues.

Metabolism

19. In laboratory animals, the major metabolic transformation route of lower chlorophenols is rapid conjugation to glucuronates and sulphates in the liver (WHO, 1989). This conjugation, in addition to dechlorination and methylation, results in detoxification. Higher chlorophenols, PCP and some TeCP congeners, appear to undergo dechlorination and/or oxidation prior to conjugation (WHO, 1989). For example, 2,3,5,6-TeCP gives rise to tetrachloro *p*-hydroquinone as a major metabolite *in vivo* whilst PCP can be converted to tetrachloro-1,2 and tetrachloro-1,4- hydroquinone *in vitro* by rat liver microsomes (van Ommen *et al.*, 1986). These hydroquinone metabolites have been shown to bind covalently to protein and DNA *in vitro* (see paragraph 160 below). Hydroquinones have been reported as minor metabolites of other TeCPs with the occurrence of very low levels of catechols being reported following exposure to 4-CP which could lead to hydroquinone formation. However, other data on whether, or to what extent, hydroquinones are formed during the metabolism of other chlorophenols *in vivo* is lacking.
20. Similar conjugation to that described above occurs in humans. In the urine of sawmill workers, 98 and 93% of the absorbed tri- and tetrachlorophenols respectively were excreted as conjugated metabolites (Pekari *et al.*, 1991). The level of conjugated PCP in the urine was lower at 76%. The study measured total and conjugated chlorophenols and it is unclear whether unconjugated chlorophenols (the difference between the two measures) represent the parent compound or other closely related metabolites. At low concentrations, sulfate conjugation was predominant but with increasing concentration, the proportion of glucuronide conjugates increased, especially for PCP.

Monochlorophenols

21. The major metabolic transformation for the lower chlorophenols is conjugation prior to clearance. This has been demonstrated in a number of studies in rabbits (Azouz *et al.*, 1953; Bray *et al.*, 1952; Spencer and Williams, 1950). In

the latter study, groups of 6 rabbits were given a single gavage dose of 171 mg/kg of 2-CP or 4-CP emulsified in water. For both isomers, the 24-hour urine analysis indicated that between 78 and 88 % of the administered dose was excreted as the glucuronide, and 12 -20 % of the dose was excreted as the ethereal sulphate. In a further experiment by the same authors, 4 rabbits were given an average dose of 395 mg/kg 4-CP as a single gavage dose. After 36 hours, 54.1% of the administered dose appeared in the urine as the glucuronide conjugate, and 10.4% of the administered dose appeared in the ethereal sulfate fraction, with 0.1% of the administered dose excreted as 4-chlorocatechol. Total recovery (64.5%) in the study was low. The excretion of low levels of chlorocatechols (1.5-4.5%) has been reported in other studies in rabbits (Azouz *et al.*, 1953).

22. Phornchirasilp *et al.*, (1989a) proposed that in mice 4-CP is metabolized by cytochrome P-450 (CYP) enzymes to intermediates that react with glutathione based on the observation that 4-CP treatment of mice depleted liver thiol stores. The depletion was prevented by the CYP inhibitor SKF 525-A. *In vitro* studies suggested that semiquinone and quinone species derived from 4-chlorocatechol could be the chemically reactive intermediates leading to the depletion of liver thiol since scavengers of superoxide anions or agents that are suggested to reduce accumulation of semiquinone and quinone species decreased binding to mouse liver microsomal proteins in contrast to epoxide hydrolase inhibitors which had no effect.

Dichlorophenols

23. Two minor metabolites of 2,4-DCP, both dichloromethoxy phenols, have been identified in studies using isolated perfused rat livers (Somani *et al.*, 1984).
24. 2,4-DCP has been shown to be metabolized into two major metabolites identified as 2-chloro-1,4- hydroxyquinone and 2-chloro-1,4-benzoquinone by microsomal fractions and whole cells of yeast *Saccharomyces cerevisiae* expressing human CYP 3A4 (Mehmood *et al.*, 1997). The metabolites were identified by TLC and GC-MS Another metabolite, 1,2,4-hydroxybenzene¹, was also detected during biotransformation by whole cells but was not observed in microsomal fractions. 2,4-DCP metabolism was dependent on NADPH in microsomal fractions and no activity was observed in the microsomal fraction or the whole cells of the control yeast. Thus, human CYP3A4 can remove either or both chlorine atoms from the aromatic ring of the 2,4-DCP molecule, forming 2-chloro-1,4-hydroxyquinone and 1,2,4-hydroxybenzene, respectively (Mehmood *et al.*, 1997). The authors proposed that first step in the pathway was the hydroxylation of 2,4-DCP to 2-chloro-1,4-

¹ As reported in the paper- it seems likely this refers to 1,2,4-trihydroxybenzene

hydroxyquinone which had previously been reported in fungi and was a metabolite expected of a CYP mediated biotransformation. Of the other metabolites, 2-chloro-1,4-benzoquinone could be a result of peroxidase metabolism of 2,4-DCP, though it was also possible that the yeast could have a dehydrogenase which could produce it. It was also suggested that the formation of 1,2,4-hydroxybenzene could be CYP mediated.

25. Similarly to monochlorophenols, dichlorophenols are largely excreted as conjugated compounds. A study in rats found that glucuronides and other unspecified conjugates were formed following a single intravenous dose of 2,4-DCP (10 mg/kg) (Somani and Khalique 1982).

Trichlorophenols

26. Bahig *et al.*, (1981) reported that in rats 2,4,6-TCP was not significantly "degraded" but underwent a degree of isomerization to other trichlorophenol isomers followed by conjugation with glucuronic acid. Male rats eliminated 63% of a gavage dose of 2,4,6-TCP in the urine. This was present as four TCP isomers (2,4,6-TCP, 2,3,6-TCP, 2,4,5-TCP and an unidentified fourth isomer). Glucuronic acid accounted for approximately 80% of the conjugates detected in the urine. However, in a similar study by Pekari *et al* (1986) 83% of the administered 2,4,6-TCP (given i.p.) was present in the blood as a glucuronide conjugate without having undergone isomerisation to other TCPs.
27. *In vitro* studies using rat liver microsomes have shown that 2,4,5-TCP can be metabolized to 3,4,6-trichlorocatechol and a dihydroxydichlorobenzene (not further characterized) (Butte *et al.*, 1988). The metabolites were identified by GC-MS. These authors reported that metabolites were also formed by dimerization resulting in a dihydroxyhexachlorobiphenyl, a dihydroxypentachlorodiphenyl ether, two hydroxypentachlorodiphenyl ethers, a hydroxyhexachlorodiphenyl ether, and a hydroxyhexachlorodioxin or hydroxyhexachlorodiphenoxinone. The possible formation of a dioxin was discussed but it was noted that the compound could also be an ethylated hydroxy-hexachlorodiphenoxinone; though as no MS reference spectra were available for this, it was not possible to decide which was the most plausible. Juhl *et al* (1989) reported the production of 3,4,6-trichlorocatechol and 2,5-dichlorohydroquinone in a similar experimental system, suggesting that the latter possibility might be more plausible.
28. Metabolites generated following incubation of 2,4,6-TCP with rat liver S-9 fraction were 2,6-dichloro-1,4-hydroquinone and two isomers of hydroxypentachlorodiphenyl ether (Juhl *et al.*, 1989). The 2,6-dichloro-1,4-semiquinone free radical was also identified.

Tetrachlorophenols

29. In rats given i.p. doses of 2,3,4,5- or 2,3,4,6-TeCP, a trichloro-p-hydroquinone was identified in the urine as a minor metabolite (Ahlborg and Larsson 1978) the rest of the dose being excreted largely unchanged. However, following treatment with 2,3,5,6-TeCP, about 35% of the recovered dose (total recovery 98.7%) was tetrachloro-p-hydroquinone, while the remaining was the unchanged parent compound.

Pentachlorophenol

30. Microsomal metabolism of PCP was reported to produce tetrachloro 1,2- and tetrachloro 1,4-hydroquinone; no other conversion products were detected (van Ommen *et al.*, 1986). Microsomes from isosafrole –pretreated rats were the most effective in catalysing the reaction, producing a 7 fold increase above control with hexachlorobenzene, phenobarbital and 3-methylcholanthrene induced rats producing a 2-3 fold increases over control; induction varied slightly between male and female rat. The two isomers were produced in different ratios by the different inducers.

31. In rats given single oral doses of PCP, tetrachlorohydroquinone (TCHQ) conjugates were detected in the urine; it was not possible to detect TCHQ in the plasma and it was suggested that it was either conjugated or oxidised to benzoquinone in the liver. (Reigner *et al.*, 1991).

Elimination and Excretion

32. In experimental animals, chlorophenols are eliminated primarily in the urine. For example, Freitag *et al.* (1982) (cited WHO, 1989) administered ¹⁴C-2,4,6-TCP to rats orally for 3 days. Within 7 days, 82.3% of the label was excreted in the urine and 22.2% in the faeces; the original reference has not been obtained and it is unclear whether or to what extent this is unabsorbed material. Bahig *et al.* (1981) found that 92.5% of a daily oral dose (25 µg by gavage for 15 days) of ¹⁴C-2,4,6-TCP was excreted by rats in the urine, while 6.4% was found in the faeces. Similarly, Ahlborg & Thunberg (1980) reported that 2,4,5-TCP given to rats was excreted rapidly (within 24 h) with very little retention by the animal. Pekari *et al.* (1986) estimated the half-times for the elimination of a 25 mg/kg bw i.p. dose of 2,4,6-TCP from the blood, liver, muscle, fat, brain, and kidney of rats to be between 1.4 and 1.8 h.

33. In a study of elimination, rats were given a 10 mg/kg bw i.v. dose of 2,4,-DCP (Somani & Khalique, 1982); the compound was eliminated most rapidly from brain tissue followed by plasma, fat, liver, and kidney with half-lives of 10, 10, 15 and 30 min respectively. A half life for overall elimination was not given.

34. In rats given single doses of 2.5 mg/kg PCP by stomach tube, clearance was essentially metabolic with only 5% of the dose being excreted unchanged

(Reigner *et al.*, 1991). It was reported that over 60% of the dose was excreted in the urine as conjugated PCP and conjugated TCHQ; around 10% of the dose was recovered in faeces as PCP and/or metabolites, suggesting that biliary excretion contributed to overall elimination; overall half-life was 7.54h. In mice given single doses of PCP by stomach tube, 8% of the dose was recovered in the urine unchanged with the majority being eliminated as conjugates (Reigner *et al.*, 1992). A proportion of the PCP was recovered as TCHQ (5%) or its conjugates (15%); overall half-life was 5.2h. As with rats, recovery of PCP and TCHQ suggested some biliary excretion had occurred.

35. Uhl *et al.* (1986) gave PCP orally to three volunteers at single doses of 3.9, 4.5, 9 and 18.8 mg. Daily urinary excretion of PCP and PCP conjugated to glucuronic acid was monitored. Assuming first-order elimination kinetics an elimination half-life of 20 days was derived. To eliminate interference from background PCP exposure, the excretion of ¹³C labelled PCP was investigated, with an elimination half-life of 17 days found in both urine and blood. The long elimination half-life of PCP was explained by the low urinary clearance due to the high plasma protein binding (greater than 96%) and tubular reabsorption. Data on elimination in animals were discussed with shorter half lives 10-121h in male rats (Braun *et al.*, 1977) and a plasma elimination half life of 72 and 83.5h in male and female macaque monkeys (Braun *et al.* 1976) being cited. It was noted by Uhl *et al.* (1986) that their work suggested much slower elimination in humans than the 90% elimination within 7 days reported by Braun *et al.*, 1977.

Chlorophenol Toxicity

36. In general, the toxicity of chlorophenols increases with an increase in the chlorination of the phenol molecule (WHO, 1989). Most mono-, di-, and tri-chlorophenols are moderately toxic when administered orally with LD50 values ranging between 230 and 4000 mg/kg body weight. In general, the less-chlorinated phenols have an acute oral toxicity very close to that of phenol (530-650 mg/kg bw). TCPs are more acutely toxic, with LD50 values between 100 and 400 mg/kg body weight (Ahlborg & Thunberg, 1980; Hattula *et al.*, 1981). Thus, the general order of decreasing acute oral toxicity is: TeCP, MCP, DCP, TCP (WHO, 1989).

Genotoxicity of chlorophenols

2- Chlorophenol

In vitro genotoxicity studies

Bacteria

37. The results of bacterial mutagenicity assays for 2-CP were primarily negative. In standard *Salmonella typhimurium* reverse mutation assays with strains TA 98, TA100, TA1535 and TA1537, 2-CP treatment at concentrations of 10-1000 µg/plate did not increase numbers of revertants in the presence or absence of activation (Haworth *et al.*, 1983). 2-CP was also negative in strain TA100 (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate in the absence of metabolic activation.
38. 2-CP either with or without S9 activation, did not show positive gene expression in an *umu* test system in *S. typhimurium* strains TA1535/pSK1002 (Ono *et al.*, 1992). It has not been possible to obtain this reference which is cited in the ATSDR review; experimental details are not given in the abstract.
39. Concentrations of 121-7,734 µM (15.6- 994 mg/L) 2-CP were negative in a prophage induction assay with *Escherichia coli* (DeMarini *et al.*, 1990) in both the presence and absence of S9 activation. This paper is attached at Annex 1.

Mammalian cells

40. A study by Önfelt, (1987) was designed to investigate the mechanism by which chemicals cause spindle disruption either in a non-specific (physical) manner by partitioning the compound into cellular hydrophobic compartments or by more specific chemical effects. Thus the chemicals tested were a mixture of those expected to act either specifically or non-specifically. The 4 chlorophenols selected were included as they were thought to uncouple oxidative phosphorylation. It was reported that 2-CP induced slight-to-moderate increases in c-mitosis (indicating disturbances of the spindle function) and aneuploidy in Chinese hamster V79 lung cells. At a concentration of 0.8mM, 12/105 cells (11.4%) had >22 chromosomes, which was statistically significant ($p < 0.025$) by the chi-square test. The number of cells with 27-41 chromosomes; number of cells with > 41 chromosomes was 3;1 and cell survival was 92%. Full results are not given but the increase in c-mitosis appears to be dose-related over a concentration range of 0.05 to 5mM. The authors stated that the optima for c-mitosis were within a non toxic interval of the concentration range. This paper is attached at Annex 1.

In vivo genotoxicity studies

41. Administration of 2-CP by stomach tube at doses up to 69 mg/kg bw/day for 14 days) had no effect on the incidence of sister chromatid exchanges (SCEs) in the testes or bone marrow of mice or on mitotic index in the bone marrow (Borzelleca *et al.*, 1985). Body weights were decreased in the top dose group,

indicating systemic toxicity was occurring. These data were published as conference proceedings and no other details are provided.

Other studies

42. Boutwell & Bosch (1959) studied the tumour-promoting action of 2-CP in 35 female Sutter mice following the single dermal application of 9,10-dimethyl-1,2-benzanthracene (DMBA) (25 µl of 0.3% DMBA in benzene) as an initiator. One week after exposure to DMBA, twice weekly applications of 25 µl of a 20% solution of 2-CP in benzene were made for 12-24 weeks. The control group received the pre-treatment dose of DMBA only. Of the surviving animals, 61% had papillomas and 10% had carcinomas compared to the controls with 7 and 0% respectively. The authors noted that the 2-CP had a tumour-promoting action similar to that of phenol.
43. Exon & Koller (1981; 1985) investigated the effects of pre, post- and combined pre and postnatal exposure of rats to 2-CP at levels of 5, 50 or 500 mg/L in drinking water. Achieved doses are not stated. Prenatal exposure occurred from 3 weeks of age to parturition with mating carried out at 3 months. Post-natal or combined exposure continued until the occurrence of tumours or 24 months of age. There was no difference in tumour incidence, latency or type between 2-CP treated rats and the controls. However, when the animals received treatment with ethylnitrosourea (ENU) given as ethylurea in feed and sodium nitrite in drinking water, 2-CP acted as a promoter of carcinogenic activity, reducing tumour latency and increasing tumour incidence in male rats exposed both pre- and post-natally, compared with the controls receiving only ENU; this effect was not seen in females. It was noted that the tumour incidence following exposure to ENU only was unexpectedly high. The group sizes (24-28) in this experiment were small and relatively limited data are provided; data on the types of tumours which occurred were not provided.

Summary/conclusions 2-CP

44. 2-CP appears to be non-mutagenic in bacterial cells, but may be an *in vitro* clastogen in mammalian cells, inducing aneuploidy. The mode of action is unknown. There are insufficient data to draw conclusions with regard to genotoxicity *in vivo*. Limited experimental data suggest that 2-CP may have some promoting activity.

3-Chlorophenol

In vitro genotoxicity studies

Bacteria

45. The results of bacterial mutation assays with 3-CP were primarily negative. In standard *S. typhimurium* reverse mutation assays with strains TA 98, TA100, TA1535 and TA1537, 3-CP at 10-1000 µg/plate did not increase numbers of revertants in the presence or absence of S9 activation (Haworth *et al.*, 1983). 3-CP was also negative in strain TA100 (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate. However, Strobel and Grummt (1987) tested concentrations of 10-1000 µg/plate 3-CP; the results are given in Table III of the paper which is attached at annex 1. Examination of the results provided suggests that an increased number of revertants were induced in *S. typhimurium* strains TA100, TA 97 and TA104 but not in TA98. The effects were most pronounced in strain TA97, in the presence and absence of metabolic activation. Statistical analyses are not provided and where increases have occurred these are less than those found in the appropriate positive controls, possibly suggesting lower activity; the mutagenic response tends to peak in the middle of the concentration range, and decrease thereafter, presumably indicating the onset of toxicity. There are no detailed comments made on the results for the individual compounds, though it is noted that 3-CP was capable of inducing both frameshift and base-pair mutations. The 3-CP was commercially obtained and it is unclear why the results differ from those of other authors.

46. Concentrations of 37.9-606 µM 3-CP were negative in a prophage induction assay with *E. coli* (DeMarini *et al.*, 1990) in the presence of activation and equivocal in the absence of S9 activation (a weak positive response was produced in one of the 2 replicate experiments).

Mammalian cells

47. No data identified.

In vivo genotoxicity studies

48. No data identified.

Other studies

49. Boutwell & Bosch (1959) studied the tumour-promoting action of 3-CP in mice following the dermal application of DMBA (25 µl of 0.3% DMBA in benzene) as an initiator. Of the surviving animals, 67% had papillomas and none had carcinomas compared to the controls which had 7 and 0% respectively. The authors noted that the 3-CP had a tumour-promoting action similar to that of phenol. See paragraph 42 above for more of the experimental details.

Summary/conclusions 3-CP

50. The majority of studies suggest that 3-CP does not appear to be mutagenic in bacterial cells. There are insufficient data to assess *in vitro* mutagenicity in mammalian cells or *in vivo* mutagenicity.

4-Chlorophenol

In vitro genotoxicity studies

Bacteria

51. The results of bacterial mutation assays with 4-CP were primarily negative. In standard *S. typhimurium* reverse mutation assays with strains TA98, TA100, TA1535 and TA1537, 4-CP at 10-1000 µg/plate did not increase numbers of revertants in the presence or absence of metabolic activation (Haworth *et al.*, 1983). In the absence of S9, 4-CP was negative in strain TA100 at concentrations of 0.1-100 µg/plate (Rapson *et al.*, 1980). A concentration of 200 µg/plate 4-CP without metabolic activation had a marginally positive response in strain TA1537, producing 172 net revertants (Seuferer *et al.*, 1979); the threshold for a mutagenic response was stated to be 100 revertants per plate and the positive control, 2,6-difluorobenzoic acid produced 321. There was no effect in strains TA98, TA100, TA1535, or TA1538. In contrast Strobel and Grummt (1987) reported that concentrations of 10-1000 µg/plate 4-CP also induced an increased number of revertants in *S. typhimurium* strains TA98, TA100, TA97 and TA104 only in the presence of S9 activation. The effects were most pronounced in strain TA97. As noted above, statistical analyses were not provided and where the increases in the number of revertants occurred these were of a lower order than the equivalent positive controls. The results are given in Table III of the paper which is attached at Annex 1.

52. 4-CP was not genotoxic in an *umu* test system at concentrations of 100-400 µg/ml (Sakagami *et al.*, 1988). The authors reported that 4-CP had previously tested positive in the Ames test in their laboratory but no further details of the latter study have been identified.

53. 4-CP was negative at concentrations of 78.4-2,424 µM in a prophage induction assay with *E. coli* (DeMarini *et al.*, 1990).

Mammalian cells

54. 4-CP has been shown to induce chromosome aberrations in Syrian Hamster Embryo (SHE) cells with exogenous metabolic activation (Hagiwara *et al.*, 2006). In the presence of metabolic activation, 9, 12, 20 and 20 % aberrant metaphases were reported at concentrations of 30, 100, 300 and 1000 μM 4-CP respectively. A range of aberrations were observed including chromatid gaps, breaks and exchanges, but no positive control data were provided. At concentrations of 30, 300 and 1000 μM respectively, 8, 12 and 18% polyploidy and endoreduplication were reported. Relative colony forming efficiency was 99 and 69% at concentrations of 300 and 1000 μM 4-CP respectively. In the absence of activation, an incidence of 1 and 5% chromosome gaps was reported at concentrations of 30 and 1000 μM 4-CP respectively, but no other chromosomal aberrations were observed; this was considered to be a negative result. The colony forming efficiency was 82 % at 1000 μM 4-CP. The 4-CP was commercially obtained and stated to be >95% pure.
55. Concentrations of 10-1000 μM 4-CP did not elicit cell transformation or unscheduled DNA synthesis (UDS) in SHE cells (Hamaguchi *et al.*, 2000; Yamaguchi *et al.*, 2003).
56. 4-CP has also been shown to be negative in the comet assay using mouse lymphoma cells and human fibroblasts (Ribeiro *et al.*, 2004). In mouse lymphoma cells, tail moment and tail intensity were 0.33 ± 0.02 and 3.02 ± 0.05 compared with 0.27 ± 0.06 and 2.99 ± 0.5 for the negative and 3.50 ± 1.23 and 22.59 ± 6.48 for the positive controls. Cell viability was approximately 90% for all treatment groups.

In vivo genotoxicity studies

57. No data identified.

Other studies

58. Chen *et al.* (2004) reported that 4-CP was able to induce apoptosis in fibroblast L929 cells in a concentration or time-dependent manner with EC50 values of 2.18 and 1.18 mmol/L at 24 and 48 h respectively.

4-CP summary and conclusions

59. 4-CP appears to be non-mutagenic in bacterial cells, but could have clastogenic effects in mammalian cells. There is insufficient evidence to assess *in vivo* genotoxicity.

2,3-dichlorophenol

In vitro genotoxicity studies

Bacteria

60. 2,3-DCP has been reported to be negative in *S. typhimurium* strains TA 98, 100, 1535 and 1537 at concentrations of 3.3 to 333 µg/plate in both the presence and absence of metabolic activation (Haworth *et al.*, 1983). Similar findings were reported by Räsänen *et al.*, (1977) at a concentration of 5 µg/plate. 2,3-DCP was also negative in strain TA100 without metabolic activation (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate.

61. It was reported that 2,3 DCP was weakly positive with activation in an *E. coli* prophage induction assay at concentrations of 19.28-154.24 µM (DeMarini *et al.*, 1990).

Mammalian cells

62. No data identified.

In vivo genotoxicity studies

63. Borzelleca *et al* (1985) reported that an i.p. dose of 2,3-DCP was negative for sister chromatid exchange. No further details are provided, it is unclear whether the work was in mice or rats, it should be noted that the majority of the studies reported in this paper were in mice.

2,3-DCP summary/conclusions.

64. 2,3-DCP does not appear to be mutagenic in bacterial cells. There are insufficient data to assess genotoxicity in mammalian cells or to assess *in vivo* mutagenicity.

2,4-dichlorophenol

In vitro genotoxicity studies

Bacteria

65. *S. typhimurium* assays of 2,4-DCP were mostly negative. Haworth *et al.* (1983) reported that concentrations of 3.3-33 µg/plate 2,4-DCP were negative in *S. typhimurium* strains TA 98, TA100, TA1535 and TA1537 in the presence and absence of metabolic activation. Similarly, a concentration of 5 µg/plate 2,4-DCP was reported to be non-mutagenic in the same strains in both the presence and absence of activation (Räsänen *et al.*, 1977). Rapson *et al.*,

(1980) reported that in the absence of S9, 4-CP was negative in strain TA100 at concentrations of 0.1-100 µg/plate. In mutagenicity studies conducted to supplement a carcinogenicity bioassay (NTP, 1989), concentrations of 3.3-33 µg/plate 2,4-DCP did not produce any revertant colonies in *S. typhimurium* strains TA 98, TA100, or TA1537 in the presence or absence of rat or hamster S9 and yielded equivocal results with TA 1535 only in the presence of hamster S9 activation. In this strain, one experimental replicate was considered equivocal with the number of revertants increasing from 8 ± 0.9 to 17 ± 0.7 , with slight toxicity being apparent, and the second, weakly positive with the number of revertants increasing from 11 ± 3.2 to 21 ± 1.5 . The replicates of the positive control (aminoanthracene) produced $1,639 \pm 36.9$ or $1,768 \pm 33$ revertants.

66. Probst *et al.*, (1981) tested over 200 chemicals as part of a comparison study with the UDS assay, and reported that 2,4 DCP was negative in a modified Ames test system in *S. typhimurium* strains G46, TA1535, TA1000, C3076, TA1537, D3052, TA 1538, and TA 98 and in 2 strains of *E. coli* WP2 and WP2 uvrA- in both the presence and absence of activation. Few details are reported but the concentration range tested was stated to be 10,000 fold.

67. It was reported that 2,4 DCP was negative with and without activation in an *E. coli* prophage induction assay at concentrations of 7.5-240 µM (DeMarini *et al.*, 1990).

68. 2,4-DCP showed positive gene expression in an *umu* test system in *S. typhimurium* strains TA1535/pSK1002 without activation but was negative with activation (Ono *et al.*, 1992). This is a secondary citation from the ATSDR review (1999); it has not been possible to obtain the original paper and no further details are given in the abstract.

Mammalian cells

69. A concentration of 50 nmol/ml 2,4-DCP did not induce unscheduled DNA synthesis in cultured rat hepatocytes (Probst *et al.*, 1981). The study was designed to compare the results of UDS assays to those of Ames tests; 218 chemicals were tested at 8 concentrations over the range 0.5- 1000 nmoles/ml. The hepatocytes were obtained by *in situ* perfusion of Fischer 1344 rats; hepatocyte viability was 86-92%. 2,4-DCP gave a nuclear silver grain count of 0.7 ± 2.2 , compared to 0.2 ± 1.4 in the controls and 9.9 ± 2.4 and 56.1 ± 8.4 for 0.5 and 50 nmoles/ml acetylaminofluorine, the positive control, respectively. It was noted that there was no dose related increase in UDS activity over the concentration range tested, but the full results are not provided.

70. Concentrations of 10-60 µg/ml 2,4-DCP increased the number of trifluorothymidine resistant cells in the mouse L5178Y assay without metabolic

activation; it was not tested with activation (NTP, 1988). In the first set of experiments, the mutant fraction was 27.5 ± 2.3 , 29.5 ± 8.5 , 27.0 ± 6.0 , 34.0 ± 6.2 , 37.0 ± 9.0 and 69.0 ± 7.5 for control (ethanol), 10, 20, 40, 50 and 60 $\mu\text{g/ml}$ 2,4-DCP respectively, in the second set the mutant fractions were 26.3 ± 3.1 , 32.7 ± 4.7 , 99.0 ± 25.0 and 163.0 ± 26.0 for control (ethanol) 20, 30, 40 and 50 $\mu\text{g/ml}$ 2,4 DCP respectively. The positive control, 5 $\mu\text{g/ml}$ methyl methanesulfonate (MMS) produced mutant fractions of 192.3 ± 7.5 and 271.3 ± 5.5 in the two experiments. Cloning efficiency was reported to be in the range 52.5 to 82.6 % at the different 2,4-DCP concentrations, compared to 83.8 and 88.3% in the controls and 58.3% for the positive control MMS. Lethality occurred at 50 $\mu\text{g/ml}$ in one of the two replicates.

71. Concentrations of 12.5 -50 $\mu\text{g/ml}$ 2,4-DCP were cytotoxic to V79 cells, but failed to produce significant increases in the frequency of 6-thioguanine (TG)-resistant mutants; S9 activation was not used (Jansson and Jansson, 1986). Survival was 108, 80 and 18% for 12.5, 25 and 50 $\mu\text{g/ml}$ 2,4-DCP with the number of TG resistant mutants being 6,6 and 0/10⁶ cells compared to 100% survival and 12 TG resistant mutants/10⁶ cells in the solvent control. The positive control, 200 $\mu\text{g/ml}$ EMS, provided the anticipated positive result producing 965 TG resistant mutants/10⁶ cells with survival of 84%. This paper is attached at Annex 1.
72. A concentration of 0.5mM 2,4-DCP produced a moderate increase in aneuploidy and polyploidy in Chinese hamster V79 cells (Önfelt 1987). At this concentration 13/103 cells (12.6%) had >22 chromosomes, which was statistically significant ($p < 0.01$) by the chi-square test. The number of cells with 27-41 chromosomes; number of cells with > 41 chromosomes was 2;2. Cell survival was 90%. Metabolic activation does not appear to have been used. The authors also reported that c-mitosis was increased; over the concentration range tested, a decrease in c-mitosis was apparent at concentrations consistent with severe toxicity meaning that fixation effect cannot be excluded.
73. *In vitro* exposure of Chinese hamster ovary (CHO) cells to 2,4-DCP significantly increased the frequency of SCEs in both the presence and absence of S9 activation (NTP, 1989). A range of concentrations (0.167 -12.6 $\mu\text{g/ml}$ in the absence of S9 and 99.7-160 $\mu\text{g/ml}$ in the presence of S9) were tested in different experiments with relative SCEs /cell% reaching a maximum of 140.7 compared to a maximum of 525% and 452% for the positive controls mitomycin c and cyclophosphamide.
74. 2,4-DCP did not cause chromosomal aberrations in CHO cells in the presence or absence of S9 activation (NTP, 1989). In the absence of activation, concentrations of 40.2, 50.3 and 75 $\mu\text{g/ml}$ 2,4-DCP produced 4, 6, and 5 aberrant cells in the 100 cells counted compared to 3/100 for the solvent

control and 22/50 for the positive control, mitomycin C. In the presence of activation, concentrations of 100.5, 125 and 150 µg/ml 2,4-DCP produced 0, 1, and 2 aberrant cells in the 100 cells counted compared to 0/100 for the solvent control and 7/50 for the positive control, cyclophosphamide. The next dose level 176 µg/ml was toxic.

In vivo genotoxicity studies

75. Groups of 20 mice/sex/dose group were given drinking water containing 0.2, 0.6 or 2 mg/ml 2,4-DCP (equivalent to 50, 150 or 5000 mg/kg bw/day) for 90 days (Borzelleca *et al.*, 1985). The treatment was negative for SCE induction in the testes and bone marrow; no further details are provided.

Other studies

76. Chen *et al.* (2004) reported that 2,4-DCP was able to induce apoptosis in fibroblast L929 cells in a concentration or time-dependent manner with EC50 values of 0.83 and 0.13 mmol/L at 24 and 48 h respectively.

77. A 2-year carcinogenicity study on 2,4-DCP was carried out in F344 rats and B6C3F1 mice by the US National Toxicology Program (NTP, 1988). Diets containing 0, 5,000 or 10,000 ppm 2,4-DCP were fed to groups of 50 male rats and 50 male and 50 female mice for 103 weeks. Groups of 50 female rats received diets containing 0, 2,500 or 5,000 ppm. Mean body weights were reduced, usually by several percent, in all of the high-dose groups as well as the low-dose groups of female mice. Mean food consumption was reduced in all treated groups by several percent in rats and in a dose-related manner in mice (up to 22%). No significant differences in the survival of any treated group occurred. There were dose-related increases in the incidence of multinucleated hepatocytes in male mice. No compound-related increases in the incidence of neoplastic lesions were observed; in fact, incidences were reduced for mononuclear cell leukaemia in male rats (both doses) and malignant lymphomas in female mice (high dose only). This study was not considered in the IARC review of 1987 where the chlorophenols were classified as group 2B. However, it was included in the revised assessment in 1999 where the classification of the chlorophenols as Group 2B was confirmed (IARC, 1999).

78. Boutwell & Bosch (1959) studied the tumour-promoting action of 2,4-DCP, in mice following a single dermal application of DMBA; see paragraph 42 for experimental details. Of the surviving animals, 48% had papillomas and 11% had carcinomas compared to the controls with 7 and 0% respectively. The authors noted that the 2-CP had a tumour-promoting action similar to that of phenol.

79. Exon & Koller (1985) investigated the effects of pre-, post- and combined pre- and post-natal exposure of rats to 2,4-DCP at levels of 3, 30 or 300 mg/L in the drinking water. See paragraph 43 for experimental details. It is stated that the carcinogenicity of 2,4-DCP was not tested, but a table is provided which indicates that 2,4-DCP did not appear to increase tumour incidence or latency compared to controls: statistical analyses are not provided. In addition, 2,4-DCP did not act as a promoter of carcinogenic activity in rats pre-treated with ENU. However, it is stated that the results of this experiment cannot be interpreted since, due to an unusually high level of tumours induced by ENU in the earlier 2-CP experiment, the ENU dose was reduced with the result that the number of tumours in the ENU treated group was not significantly different to the untreated controls. The authors considered it was possible that most or all of the tumours in the 2,4-DCP group were spontaneous. It should be noted that the group sizes in this experiment were small and relatively limited data are provided; data on the types of tumours occurring are not provided.

2,4-DCP summary/conclusions

80. 2,4-DCP does not appear to be mutagenic in bacterial cells but may have clastogenic effects in mammalian cells *in vitro*. There are insufficient data to assess *in vivo* genotoxicity.

2,5-dichlorophenol

In vitro genotoxicity studies

Bacteria

81. A concentration of 5 µg/plate 2,5-dichlorophenol was non-mutagenic in *S. typhimurium* strains TA98, TA100, TA 1535 and TA 1537 in the presence and absence of metabolic activation (Räsänen *et al.*, 1977). Similarly, 3.3-33 µg/plate 2,5-DCP was reported to be negative in the same strains (Haworth *et al.*, 1983). 2,5-DCP was also reported to be negative in strain TA100 without metabolic activation (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate.

82. It was reported that 2,5-DCP was weakly positive with activation in an *E. coli* prophage induction assay at concentrations of 19.28-154.24 µM (DeMarini *et al.*, 1990).

Mammalian cells

83. CHO/HPRT-gene mutation tests yielded negative results for 2,5-DCP with and without metabolic activation (Tegethoff *et al.*, 2000). In the absence of S9, the

mutant frequencies were 16.2, 21.3, 19.0, 20.7, and 22.5/10⁶ cells at doses of 100, 125, 150, 200 and 250 µg/ml respectively compared to 14.9 and 106.8/10⁶ cells in the solvent and positive (5-bromo-2-deoxyuridine) controls. In the presence of S9, the mutant frequencies were 12.6, 12.1, 7.8, 9.8, 11.2 and 6.5/10⁶ cells at doses of 62.5, 75, 100, 125, 150 and 200 µg/ml respectively compared to 6.2 and 151.7/10⁶ cells in the solvent and positive (3-methylcholanthrene) controls. Relative survival over the treated concentration range was 89.5-20.1% and 77-6.9% in the absence and presence of S9 respectively.

In vivo genotoxicity studies

84. 2,5-DCP was negative in a bone marrow micronucleus assay in which NMRI mice (5 males and 5 females/treatment group) were given a single dose of 1500 mg/kg bw by gavage and the animals were sacrificed 24, 48 and 72 hours after treatment (Tegethoff *et al.*, 2000). 1000 polychromatic erythrocytes (PCEs) were scored per animal; the number of micronucleated cells/1000 PCEs was 0.6 ± 0.8, 1.4 ± 1.2 and 0.9 ± 1.0 at 24, 48 and 72 h compared to 0.7 ± 0.8 and 13.0 ± 4.9 in the solvent and positive (cyclophosphamide) controls respectively. The dose used was stated to be close to the LD50 and it was noted that there was a reduction in the percentage of polychromatic erythrocytes amongst all erythrocytes demonstrating that appropriate exposure to 2,5-DCP had occurred. This is a well conducted study which appears to be broadly consistent with OECD guidelines.

2,5-DCP summary/conclusions

85. 2,5-DCP is not mutagenic in bacterial or mammalian cells *in vitro* and was negative in an *in vivo* mouse micronucleus test.

2,6-dichlorophenol

In vitro genotoxicity studies

Bacteria

86. A concentration of 5 µg/plate 2,6-dichlorophenol was non-mutagenic in the Ames *Salmonella* test strains TA98, TA100, TA 1535 and TA 1537 in both the presence and absence of activation (Räsänen *et al.*, 1977). Concentrations of 3.3-33 and 0.1 mg/plate 2,5-DCP were also reported to be negative in the same strains with and without activation (Haworth *et al.*, 1983; Nestmann *et al.*, 1980); the latter authors also reported a negative response in strain TA1358. 2,6-DCP was also negative when tested for mutagenicity using *S. typhimurium*

strain TA100. The concentrations tested were 1000, 100, 10, 1 and 0.1 µg/plate (Rapson *et al.*, 1980).

87. It was reported that 2,6-DCP was weakly positive with activation and negative without activation in an *E.coli* prophage induction assay at concentrations of 19.28-154.24 µM (DeMarini *et al.*, 1990).

Mammalian cells

88. Concentrations of 125 -500 µg/ml 2,6-DCP were cytotoxic to Chinese hamster V79 cells, but failed to produce significant increases in the frequency of 6-thioguanine-resistant mutants; S9 activation was not used (Jansson and Jansson, 1986). Survival was 88, 82 and 17% for 125, 250 and 500 µg/ml 2,6-DCP with the number of TG resistant mutants being 0, 0 and 6/10⁶ cells compared to 100% survival and 4 TG resistant mutants/10⁶ cells in the solvent control. The positive control, 200 µg/ml EMS, produced 584 TG resistant mutants/10⁶ cells with survival of 62%. Concentrations of 125 -500 µg/ml 2,4-DCP were also reported to be negative by Hattula and Knuutinen (1985) in both the direct and rat hepatocyte mediated assay methods for the production of TG resistance in V79 cells.

In vivo genotoxicity studies

89. After 1 and 2 week administration of 2,6-DCP to rats, aberrations of bone marrow chromosomes and inhibition of mitoses were observed, respectively (Chung, 1978). This study is reported on the Hazardous Substances Databank website only and information on dose and route of exposure are not given. The original study, published in 1978, is in Korean with no abstract being available.

2,6-DCP Summary/conclusion

90. 2,6-DCP does not appear to be mutagenic in bacteria cells. There are insufficient data to assess mutagenicity in mammalian cells *in vitro* or to assess *in vivo* mutagenicity.

3,4-dichlorophenol

In vitro genotoxicity studies

Bacteria

91. Concentrations of 5 and 2-200 µg/plate 3,4-dichlorophenol were non-mutagenic in the Ames *Salmonella* test strains TA98, TA100, TA1535 and TA1537 in both the presence and absence of activation (Räsänen *et al.*, 1977; Haworth *et al.*, 1983). 3,4-DCP was also reported to be negative in strain

TA100 without metabolic activation at concentrations of 0.1-100 µg/plate (Rapson *et al.*, 1980).

92. It was reported that 3,4 DCP was negative with and without activation in an *E.coli* prophage induction assay at concentrations of 9.64-154.24 µM (DeMarini *et al.*, 1990).

Mammalian cells

93. No data identified.

In vivo genotoxicity studies

94. No data identified.

3,4-DCP summary/conclusion

95. 3,4-DCP does not appear to be mutagenic in bacteria cells. There are insufficient data to assess mutagenicity in mammalian cells *in vitro* or to assess *in vivo* mutagenicity.

3,5-dichlorophenol

In vitro genotoxicity studies

Bacteria

96. Concentrations of 5 and 2.2-200 µg/plate 3,5-dichlorophenol were non-mutagenic in the Ames *Salmonella* strains TA98, TA100, TA1535 and TA1537 in both the presence and absence of activation (Räsänen *et al.*, 1977; Haworth *et al.*, 1983). 3,4-DCP was also reported to be negative in strain TA100 without metabolic activation (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate.

97. It was reported by DeMarini *et al.*, (1990) that 3,5-DCP was weakly positive in the presence of metabolic activation and negative in the absence of metabolic activation in an *E.coli* prophage induction assay at concentrations of 4.82-154.24 µM.

Mammalian cells

98. A concentration of 0.1-0.8 mM 3,5-DCP did not increase aneuploidy and polyploidy in Chinese hamster V79 cells (Önfelt 1987). S9 activation was not used. At concentrations of 0.01, 0.02 and 0.08 mM, 5/101 (4.9%) 6/101 (5.9%) and 6/100 (6%) cells respectively had >22 chromosomes, which was not statistically significant by the chi-square test, compared to 3-7% in the

controls). The number of cells with 27-41 chromosomes; number of cells with > 41 chromosomes was 3;1, 1;1 and 0;1 and cell survival was 92%. The authors also reported that c-mitosis was increased; over the concentration range tested, a subsequent decrease in c-mitosis was apparent at concentrations consistent with severe toxicity meaning that fixation effect could not be excluded.

In vivo genotoxicity studies

99. No data identified.

3,5-DCP summary/conclusion

100. 3,5-DCP does not appear to be mutagenic in bacteria cells. There are insufficient data to assess mutagenicity in mammalian cells *in vitro* or to assess *in vivo* mutagenicity.

2,3,4-trichlorophenol

In vitro genotoxicity studies

Bacteria

101. 2,3,4-TCP was reported to be negative in *Salmonella* strain TA100 without metabolic activation (Rapson *et al.*, 1980) at concentrations of 0.1-100 µg/plate. No evidence of toxicity was apparent at this dose level.

102. DeMarini *et al.*, (1990) reported that 2,3,4-TCP was positive in the presence of metabolic activation and weakly positive in the absence of activation in an *E. Coli* prophage induction assay at concentrations of 1.99-31.88 µM.

Mammalian cells

103. No data identified.

In vivo genotoxicity studies

104. No data identified.

2,3,4-TCP summary/conclusion

105. 2,3,4 TCP does not appear to be mutagenic in bacteria cells , though the data available are more limited than for other congeners. There are insufficient data to assess mutagenicity in mammalian cells *in vitro* or to assess *in vivo* mutagenicity.

2,3,5-trichlorophenol

In vitro genotoxicity studies

Bacteria

106. A concentration of 5 µg/plate 2,3,5-TCP was non-mutagenic in the Ames *Salmonella* test strains TA98, TA100, TA1535 and TA1537 in both the presence and absence of activation (Räsänen *et al.*, 1977). 2,3,5-TCP was also reported to be negative in strain TA100 without metabolic activation at concentrations of 0.1-100 µg/plate (Rapson *et al.*, 1980).
107. DeMarini *et al.*, (1990) reported that 2,3,5-TCP was equivocal in the presence of metabolic activation and negative in the absence of activation in an *E.coli* prophage induction assay at concentrations of 3.99-127.50 µM.

Mammalian cells

108. No data identified.

In vivo genotoxicity studies

109. No data identified.

2,3,5-TCP summary/conclusions

110. 2,3,5-TCP does not appear to be mutagenic in bacteria cells. There are insufficient data to assess mutagenicity in mammalian cells *in vitro* or to assess *in vivo* mutagenicity.

2,3,6-Trichlorophenol

In vitro genotoxicity studies

Bacteria

111. A concentration of 5 µg/plate 2,3,6-TCP was non-mutagenic in the Ames *Salmonella* strains TA98, TA100, TA 1535 and TA 1537 in both activated and non-activated systems (Räsänen *et al.*, 1977). 2,3,6-TCP tested negative for mutagenicity in *Salmonella* assays (NTP, 1987a); this is cited on the HSDB website, with the reference given as an NTP annual plan; no further details have been identified. 2,3,6-TCP was tested for mutagenic activity in strains TA97, TA98, TA100 and TA104 in the Ames test at concentrations of 10-1000 µg/plate (toxicity was observed in the top doses for some strains only) (Strobel and Grummt, 1987). It was reported that 2,3,6-TCP was positive in

strain TA98 in the presence of S9 only. Statistics are not provided but the increase in the number of revertents was approximately 25% of the number produced by the positive control AAF. The data are given in Table III of the paper attached at Annex 1.

112. DeMarini *et al.*, (1990) reported that 2,3,6-TCP was positive in the presence of metabolic activation only in an *E.coli* prophage induction assay at concentrations of 15.94-2550 µM.

Mammalian cells

113. 2,3,6-TCP induced an increase in chromosome aberrations in Chinese hamster ovary cells and V79 cells both with and without metabolic activation (Armstrong *et al.*, 1993) at concentrations of 400-600 µg/ml. Full results are not given but it is stated that the induction of aberrations was similar to that seen for 2,4,6-TCP and that cell numbers decreased to 69-42% of controls. From figure 43 in this paper, approximately 60% aberrations were observed at the top dose level. For more details of this study which focussed on 2,4,6-TCP see paragraph 131 below. This paper is attached at Annex 1.
114. 2,3,6-TCP tested positive for chromosome aberrations and negative results were obtained for SCEs in CHO cells (NTP, 1987b). As above, this is noted on the HSDB website, with the reference given as an NTP annual plan; further details have not been identified.

In vivo genotoxicity studies

115. No data identified.

2,3,6-TCP summary/conclusions

116. 2,3,6-TCP does not appear to be mutagenic in bacterial cells. There is some evidence of clastogenicity in mammalian cells *in vitro* but there are insufficient data to assess *in vivo* mutagenicity.

2,4,5-Trichlorophenol

In vitro genotoxicity studies

117. In a study by Juhl *et al.*, (1991) 2,4,5 TCP was incubated with rat liver S9; the metabolites were reported to produce DNA strand breaks in PM2 DNA. Among the metabolites produced were 3,4,6-trichlorocatechol and 2,5 dichlorohydroquinone, the latter being 100 times more effective at inducing strand breaks; semiquinone radicals were identified for both metabolites. The authors reported that experiments with scavengers demonstrated that the DNA

damage was mediated by reactive oxygen species but that differences in the results for the two metabolites suggested that the specific active species might be different.

Bacteria

118. 2,4,5-TCP was negative in standard *S. typhimurium* strains TA 98, TA 100, TA102 and TA104 at concentrations of 1-7.5 mg/plate in the presence and absence of activation (George *et al.*, 1992) and also in strains TA98, TA100, TA 1535 and TA 1537 with and without activation at a concentration of 5 µg/plate (Räsänen *et al.*, 1977). Similar results were reported using 2,4,5-TCP concentrations of 1-66 and 50 µg/plate (Haworth *et al.*, 1983; Nestmann *et al.*, 1980); the latter authors also reported a negative response in strain TA1358. However, 2,4,5-TCP was tested for mutagenic activity in strains TA97, TA98, TA100 and TA104 at concentrations of 10-1000 µg/plate by Strobel and Grummt (1987) and 2,4,5-TCP was reported to be positive in strain TA97 in the presence and absence of S9 and TA100 in the absence of S9. Statistics were not provided, the increase in the number of revertants was approximately 30% and 100% of the number produced by the positive controls Nor-HN2 and DNOC respectively. Toxicity was observed in the top 2 doses for all strains and at 0.25 mg/plate for some strains only. The data are given in Table III of the paper in Annex 1.
119. 2,4,5-TCP either with or without S9 activation, did not show positive gene expression in an *umu* test system in *S. typhimurium* strains TA1535/pSK1002 (Ono *et al.*, 1992). This is cited in the ATSDR review with no further details given in the abstract; it has not been possible to obtain the original.
120. DeMarini *et al.*, (1990) reported that 2,4,5-TCP was positive in the presence of S9 activation and weakly positive without activation at a concentration of 3.99-63.75 µg/plate in prophage induction assays. In the same assay performed by George *et al.*, (1992) 2,4,5-TCP was positive in the presence of S9 activation only, at concentrations of 0.4-3.2 µM.

Mammalian cells

121. Concentrations of 6.25 -50 µg/ml 2,4,5-TCP were cytotoxic to V79 cells, but failed to produce significant increases in the frequency of 6-thioguanine-resistant mutants; S9 activation was not used (Jansson and Jansson, 1986). The number of TG-resistant mutants was 3, 6, 5, 7/10⁶ cells at concentrations 6.25, 12.5, 25, and 50 respectively compared to 9/10⁶ cells in the negative control. The positive control, 200 µg/ml EMS, produced 1262 TG-resistant mutants per/10⁶ cells. Cell survival was 90, 63, 45, and 17% respectively in the 2,4,5-TCP treated cells and 64% in the EMS treated cells.

122. 2,4,5-TCP induced a small increase (up to about 10.5%) in chromosome aberrations in CHO and V79 cells both with and without metabolic activation (Armstrong *et al.*, 1993). The concentrations used (140-160 µg/ml) were associated with significant toxicity, with the cell counts being 59-49 % of controls. See paragraph 131 below for more details of this study which was focussed on 2,4,6-TCP.

In vivo genotoxicity studies

123. 2,4,5-TCP did not increase DNA damage in rats given two oral doses 1/5 of the LD50 (stated to be 164 mg/kg by ATSDR) as indicated by alkaline elution of DNA from white blood cells and liver (Kitchin and Brown, 1988). Only the abstract of this study has been identified.

Other studies

124. Boutwell & Bosch (1959) studied the tumour-promoting action of 2,4,5-TCP, in mice following the dermal application of DMBA as an initiator. See paragraph 42 for experimental detail. None of the surviving animals, had papillomas or carcinomas compared to 7 and 0% in the controls.

2,4,5-TCP summary/conclusions

125. 2,4,5-TCP does not appear to be mutagenic in bacterial cells. There is some evidence of clastogenicity in mammalian cells *in vitro* but there are insufficient data to assess *in vivo* mutagenicity.

2,4,6-Trichlorophenol

In vitro genotoxicity studies

126. 2,4,6 TCP was incubated with rat liver S9 with three metabolites identified as 2,6 dichloro-1,4-hydroquinone and two isomers of hydroxypentachlorodiphenyl being produced (Juhl *et al.*, 1989). Incubation of the metabolites with PM2 DNA produced single strand breaks. The damage was considered to be mediated by reactive oxygen species produced during the formation of a semiquinone radical.

Bacteria

127. Concentrations of 5 and 2.2-200 µg/plate 2,4,6-TCP were non-mutagenic in Salmonella strains TA98, 100, 1535 and 1537 in both the presence and absence of S9 (Räsänen *et al.*, 1977 ; Haworth *et al.*, 1983). Kinae *et al.* (1981) reported that 2,4,6-TCP was negative in strains TA 98, TA100, and TA1537 with and without exogenous metabolic activation, but was

positive in a *B. subtilis* recombination assay (Ono *et al*, 1992). This is cited in the ATSDR review but it has not been possible to obtain the original. Rapson *et al.*, (1980) reported that in the absence of S9, 2,4,6-TCP was negative in strain TA100 at concentrations of 0.1-100 µg/plate. However, at concentrations of 10-1000 µg/plate, 2,4,6-TCP was reported to be positive in strain TA97, 98 and 104 in the presence of S9. Statistics were not provided but the increase in the number of revertents was approximately 20, 50 and 15% of the number produced by the positive controls AAF and AF respectively (Strobel and Grummt, 1987). Toxicity was observed in the top dose for all strains and at 0.5 and 0.25 mg/plate for some strains only. The data are given in Table III of the paper attached at Annex 1.

128. DeMarini *et al.*, (1990) reported that 2,4,6-TCP was positive in the presence of S9 activation and weakly positive without activation at a concentration of 3.99-63.75 µg/plate in *E.coli* prophage induction assays.

Yeast cells

129. At a concentration of 400 mg/L, 2,4,6-TCP caused a weak but significant increase in the frequency of forward mutations, but did not affect intergenic or intragenic re-combinations in the MP-1 strain of the yeast *Saccharomyces cerevisiae* (Fahrig *et al.*, 1978).

Mammalian cells

130. Concentrations of 12.5 -100 µg/ml 2,4,6-TCP were cytotoxic to Chinese hamster V79 cells, but failed to produce significant increases in the frequency of 6-thioguanine-resistant mutants without metabolic activation (Jansson and Jansson, 1986). The number of TG-resistant mutants was 3, 4, 2 and 0/10⁶ cells at concentrations of 12.5, 25, 50 and 100 µg/ml respectively compared to 2/10⁶ cells in the negative control. The positive control, 200 µg/ml EMS, produced 631 TG-resistant mutants per/10⁶ cells. Cell survival was 85, 79, 72 and 53% respectively in the 2,4,5-TCP treated cells and 79% in the EMS treated cells. In a later study by the same authors (Jansson and Jansson, 1992) 2,4,6-TCP was again negative in this assay at doses of 10-180 µg/ml, producing mutant frequencies of 7-9 and 12-5 per 10⁶ clonable cells over the concentration range tested in the two replicates, compared with 13 and 8 per 10⁶ clonable cells in the solvent control. Relative survival decreased over the dose range, being as low as 14% at the top dose tested. EMS, the positive control produced 785 and 982 mutants per 10⁶ clonable cells in the two replicate experiments with relative survival of 71 and 83%. This paper is attached at Annex 1. However Hattula and Knuutinen (1985) using the direct method (not incubating with fibroblasts) reported a small increase in mutants at concentrations of 10-60 µg/mg, this was about one tenth of the mutagenic activity shown by the positive control MMNG. The number of TG-resistant

mutants was 1, 13, 53, 25 and 11/10⁶ cells at concentrations of 10, 20, 30, 45 and 60 µg/ml respectively compared to 0/10⁶ cells in the negative control. The positive control, 0.5 and 1.0 µg/mg MMNG, produced 471 and 799 TG-resistant mutants per/10⁶ cells respectively. Cell survival was 58.0, 55.0, 55.1, 47.5 and 40.5% respectively in the 2,4,6-TCP treated cells, 61.7% in the negative control and 54.5 and 45.5% in the MMNG treated cells respectively. This paper is attached at Annex 1.

131. 2,4,6-TCP did not produce chromosome aberrations or sister chromatid exchange in Chinese hamster ovary cells with or without S9 activation (Galloway *et al.*, 1987). In this screening study of 108 chemicals, 2,4,6-TCP was tested at concentrations of 5-50 and 16-500 µg/ml for SCEs in the absence and presence of S9 and 50-500 µg/ml for chromosome aberrations. No other details are provided for this compound. In contrast, Jansson and Jansson (1992) reported that 2,4,6-TCP produced a significant dose-related increase in hyperdiploidy and micronuclei in the absence of metabolic activation, suggesting that error in chromosomal segregation was the major genotoxic mechanism of 2,4,6-TCP. In this study, the cells were treated with 10-90 µg/ml 2,4,6-TCP for 24 hours and immediately sampled; with no increase in chromosomal aberrations being found. There was an increase in hyperdiploid cells and in micronuclei (cells were harvested 24 hours after the treatment period). In the first experiment there were 11, 18, 31, 34, 42 and 17 hyperdiploid cells in the solvent (DMSO), 10, 30, 60 and 90 µg/ml 2,4,6-TCP and EMS treated cells and 32, 44, 56, 88, 116 and 126 cells with micronuclei per 2000 cells. In experiment 2, there were 8, 14, 33, 32, 36 and 12 hyperdiploid cells in the solvent (DMSO), 10, 30, 60 and 90 µg/ml 2,4,6-TCP and EMS treated cells and 36, 46, 69, 77, 114 and 112 cells with micronuclei per 2000 cells scored. Armstrong *et al.*, (1993) reported that an increase in aberrations was not observed while using the protocol of Jansson and Jansson (1992) but that 2,4,6-TCP did test positive for chromosomal aberrations (deletions and exchange) following a 24 hour treatment and a 4-12h recovery period both with and without metabolic activation, producing up to 21% aberrant cells. Positive results were also obtained by using a 3h treatment and a 17h sampling period. For this protocol, the % of aberrant cells was 1.8, 1.5, 1.5, and 33.0 for control, 300, 400 and 500 µg/ml 2,4,6 TCP in the absence of S9 with cell counts in the treated cells being 75, 63 and 47% of the control value respectively. In the presence of S9 the % of aberrant cells was 2.8, 0.0, 3.5, and 66.0 for control, 300, 400 and 500 µg/ml 2,4,6 TCP with cell counts in the treated cells being 83, 81 and 42% of the control value respectively. The results were similar in CHO and V79 cells. Hyperdiploidy was also observed in V79 cells but in 12/200 cells at 100 µg/ml, lower than the incidence of 36-42/200 at 90 µg/ml reported by Jansson and Jansson *et al* (1992). In discussing the differences in the reported findings for 2,4,6-TCP, it was

suggested that the negative results reported by other authors (Galloway *et al.*, 1987; Ishidate *et al.*, 1987 –NB the latter reference was not obtained) might be due to inappropriate protocols and argued that while hyperdiploidy had been demonstrated, 2,4,6-TCP was clearly clastogenic in CHO and V79 cells and thus should be detectable in a standard test battery, without requiring specific tests for aneuploidy.

132. In the mouse L5178Y assay (without metabolic activation), trifluorothymidine resistance was increased by 2,4,6-TCP exposure (McGregor *et al.* 1988). 2,4,6-TCP treatment produced average mutant frequencies of 31, 36, 27, 42, 42, and 75 in trial 1 and 41, 51, 58, 60, 81 and 185 at concentrations of 0, 6.25, 12.5, 25, 50, and 100 respectively. The lowest observed effective dose level LOED was noted to be 80 µg/ml and the relative total growth at that dose level, 23%. The positive controls EMS and MMS produced mutant frequencies of 142 and 102 in trial 1 and 237 and 173 in trial 2.

In vivo genotoxicity studies

133. Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster* fed 2,4,6-TCP in a sucrose solution (Valencia *et al.*, 1985).
134. In a mouse spot test, pregnant mice were injected intraperitoneally with 50 or 100 mg purified 2,4,6-TCP/kg body weight on day 10 of gestation. Examination of offspring revealed an increased frequency of coat spots (reported to be 0.6% in the 50 mg/kg group versus 0.1% in controls), indicative of a weak mutagenic response (Fahrig *et al.*, 1978). At 100 mg/kg the frequency can be calculated to be 0.6%. The calculation of frequency included spots considered to be “of genetic relevance.”
135. 2,4,6-TCP did not increase DNA damage in rats given two oral doses 21 and 4 hours before sacrifice, as indicated by alkaline elution of DNA from white blood cells and the liver (Kitchin and Brown, 1988). The dose given was 1/5th of the LD50. It has not been possible to obtain the original study.

Other studies

136. Innes *et al.* (1969) tested 120 pesticides and industrial chemicals for tumorigenicity in male and female mice. Two different strains (C57BL/6 x C3H/Anf) F₁ and (C57BL/6 x AKR)F₁ were used with 18 mice of each sex in each strain. The individual compounds were administered at the maximum tolerated dose by stomach tube, from 7 days of age to 4 weeks old, and then in the diet at approximately the same dosage. The 100 mg/kg daily dose of Omal or Dowicide 2S (2,4,6-TCP) increased tumour incidence in the treated animals at the end of 72 weeks. The authors recommended additional

statistical evaluation and/or studies before a meaningful interpretation could be made. It was noted that the principal tumours were in the liver, lung and lymphoid system but few data are provided on the types of tumours associated with individual compounds.

137. A long-term oral exposure study was carried out on rats and mice to test the carcinogenicity of 2,4,6-TCP (NCI, 1979). Groups of 50 male and female F344 rats were given feed containing 5000 and 10 000 mg/kg 2,4,6-TCP for 106 weeks. Male B6C3F1 mice were dosed at the same levels as the rats for 105 weeks. Female mice were initially given dietary levels of 10 000 and 20 000 mg/kg for 38 weeks, which were then reduced to 2500 and 5000 mg/kg, respectively, for the remaining 67 weeks of the study, because they showed a marked reduction in weight gain; 50 animals per sex were treated per dose group. At the end of the study, the treated male rats showed a significantly higher dose-related incidence of malignant lymphomas and leukaemias. Leukocytosis and monocytosis of the peripheral blood and hyperplasia of the bone marrow were found in those that did not show lymphomas and leukaemias. No lymphomas and/or leukaemias were detected in female rats, but leukocytosis and monocytosis of peripheral blood and bone-marrow hyperplasia were evident. Both male and female mice displayed a dose-related statistically significant increased incidence of both hepatocellular carcinomas and adenomas. It was concluded that 2,4,6-trichlorophenol was carcinogenic for male Fisher rats and both sexes of B6C3F1 mice under the assay conditions used. The compound was obtained commercially and the purity was reported to be 96-97% with 17 minor contaminants identified by Gas Chromatography (GC). The chlorinated dibenzo-p- dioxin content was not determined. Therefore it is unclear whether any of the findings could be attributable to dioxin.

138. Boutwell & Bosch (1959) reported that 2,4,6-TCP treatment did not promote tumour formation in mice following initiation with DMBA . See paragraph 42 for experimental details.

2,4,6-TCP summary/conclusions

139. 2,4,6 TCP does not appear to be mutagenic in bacterial cells but there is evidence for clastogenicity in mammalian cells. There is insufficient evidence to assess *in vivo* mutagenicity.

3,4,5-Trichlorophenol

In vitro genotoxicity studies

Bacteria

140. 3,4,5-TCP tested negative for mutagenicity in Salmonella assays (NTP, 1987a). No further details are available. This is cited as a NTP Annual plan and it has not been possible to obtain any further details.

Mammalian cells

141. 3,4,5-TCP tested negative for chromosome aberrations and sister chromatid exchanges in CHO cells (NTP, 1987b). As noted above, it has not been able to obtain further details.
142. 3,4,5-TCP induced slight-to-moderate increases in c-mitosis (indicating disturbances of the spindle function) and aneuploidy in cultured Chinese hamster V79 lung cells (Önfelt, 1987). Metabolic activation does not appear to have been used. The increase in aneuploidy, compared to controls, was statistically significant ($p < 0.005$) by the chi-square test. At concentrations of 1.5 and 3 μM , 6/103 (5.8 %) and 17/84 (20.2%) cells respectively had >22 chromosomes, the lower dose was not statistically significant by the chi-square test, compared to 3-7% in the controls, but the higher dose was significant $p < 0.005$. The number of cells with 27-41 chromosomes; number of cells with > 41 chromosomes was 0;0 and 1;1 with cell survival of 73%. The authors also reported that c-mitosis was increased, with the optima for c-mitosis being in a non-toxic concentration interval.

In vivo genotoxicity studies

143. No data identified.

3,4,5-TCP summary/conclusions

144. There are insufficient data to draw any conclusions on 3,4,5-TCP

2,3,4,5-Tetrachlorophenol

In vitro genotoxicity studies

Bacteria

145. 2,3,4,5-TeCP was reported to be negative in strains TA 97, TA98, TA100 and TA1535 with and without metabolic activation by Zeiger *et al.*, (1988). Only the abstract of this study has been obtained.

146. DeMarini *et al.*, (1990) reported that 2,3,4,5-TeCP was positive in the presence of S9 activation at a concentration of 0.89-13.59 µg/plate in an *E. Coli* prophage induction assays.

Mammalian cells

147. No data identified.

In vivo genotoxicity studies

148. No data identified.

2,3,4,5-TeCP summary/conclusions

149. There are insufficient data to draw any conclusions on 2,3,4,5-TeCP

2,3,4,6-Tetrachlorophenol

In vitro genotoxicity studies

Bacteria

150. A concentration of 5 µg/plate 2,3,4,6-TeCP has been reported to be non-mutagenic in strains TA98, TA100, TA1535 and TA1537 in the presence and absence of activation (Räsänen *et al.*, 1977). Similar results were reported in strains TA 97, TA98, TA100 and TA1535 by Zeiger *et al.*, (1988). Only the abstract of this study has been obtained.
151. 2,3,4,6-TeCP tested positive in an *E.coli* prophage induction assay (DeMarini *et al.*, 1990), as well as being positive both with and without activation in a *umu* test system (Ono *et al.*, 1992). This is cited by ATSDR, the original has not been obtained.

Mammalian cells

152. Hattula & Knuutinen (1985) reported that purified 2,3,4,6-TeCP was weakly mutagenic in V-79 Chinese hamster cells *in vitro* at concentrations of 3.5-20 µg/mg, in the absence of metabolic activation. The number of TG resistant mutants/10⁶ cells at 3.5, 7, 10, 15 and 20 µg/mg 2,3,4,6-TeCP was 12, 17, 35, 18 and 10 compared to 0/10⁶ in the solvent control and 471 and 799 at concentrations of 0.5 and 1.0 µg/mg MNNG. Thus the maximum number of mutants produced was less than 5% of that produced by the positive control. Cloning efficiency ranged from 75.3 to 60.0 % compared to 74.3% in the negative control and 45-54% in the positive control. In the presence of a hepatocyte co-culture, 10 µg/ml 2,3,4,6-TeCP was not mutagenic, producing no TG- resistant mutants compared to 102-347 at

concentrations of 0.3-3mM N-nitrosodimethylamine (DMN). Jansson and Jansson (1986) reported that concentrations of 12.5-100 µg/ml 2,3,4,6-TeCP were cytotoxic but negative for mutation in V79 cells. A concentration of 12.5, 25, 50, and 100 µg/ml 2,3,4,6-TeCP produced 7, 16, 7 and 9 TG-resistant mutants/10⁶ cells compared to 1/10⁶ in the negative control and 1181/10⁶ in the positive control (200 µg/ml EMS). Relative survival ranged from 101-10% compared to 88% in the controls.

In vivo genotoxicity studies

153. 2,3, 4,6-TeCP did not increase DNA damage in rats given two oral doses (stated to be 1/5 of the LD50) as indicated by alkaline elution of DNA from white blood cells and the liver (Kitchin and Brown, 1988). Only the abstract of this study was available.

2,3,4,6-TeCP summary/conclusion

154. 2,3,4,6-TeCP does not appear to be mutagenic in bacterial cells, there is limited evidence of mutagenicity in mammalian cells. There is insufficient data to assess *in vivo* mutagenicity.

2,3,5,6-Tetrachlorophenol

In vitro genotoxicity studies

Bacteria

155. 2,3,5,6-TeCP was negative in strains TA 97,TA98, TA100 and TA1535 in the presence and absence of activation (Zeiger *et al.*, 1988). Only the abstract of this study is available.
156. 2,3,5,6-TeCP was weakly positive in an *E.coli* prophage induction assay at concentrations of 13.59 -108.68 µM in the presence of S9 activation and equivocal in the absence of activation (DeMarini *et al.*, 1990), as well as being positive both with and without activation in a *umu* test system in *Salmonella* (Ono *et al.*, 1992). This is cited by ATSDR, the original has not been obtained.

Mammalian cells

157. No data identified.

In vivo genotoxicity studies

158. No data identified.

2,3,5,6-TECP summary/conclusions

159. There are insufficient data available to draw any conclusions on 2,3,5,6-TeCP.

Pentachlorophenol

In vitro genotoxicity studies

In vitro studies

160. Van Ommen *et al.*, (1986) reported that PCP was able to bind to calf thymus DNA *in vitro* in the presence of rat microsomal protein; these microsomes converted PCP into the metabolites 1,4 and 1,2-TCHQ. The degree of binding to DNA was lower than that to protein. Further investigation of the protein binding (Van Ommen *et al.*, 1986) suggested that binding of the PCP metabolite TCHQ was largely dependent on the formation of reactive oxygen species.

Bacteria

161. Concentrations of 0.3 to 30 µg/plate PCP were non-mutagenic in Salmonella strains TA98, TA100, TA1535 and TA1537 in both the presence and absence of S9 (Haworth *et al.*, 1983).
162. PCP was positive in an *E.coli* prophage induction assay at concentrations of 1.47 -93.98 µM in the presence of S9 activation and weakly positive in the absence of activation (DeMarini *et al.*, 1990).

Yeast cells

163. At a concentration of 400 mg/L, PCP caused a significant increase in the frequency of forward mutations and intragenic re-combinations in the MP-1 strain of the yeast *Saccharomyces cerevisiae* (Fahrig *et al.*, 1978).

Mammalian cells

164. The induction of mutation of the hypoxanthine-guanine phosphoribosyl transferase locus and cytotoxicity of PCP was examined in V79 Chinese hamster cells without exogenous metabolic activation. PCP was shown to be cytotoxic to V79 cells, but failed to produce significant increases in the frequency of 6-thioguanine-resistant mutants (Jansson and Jansson 1986). A concentration of 6.25, 12.5, 25 and 50 µg/ml 2,3,4,6-TeCP produced 10, 0, 0 and 7 TG-resistant mutants/10⁶ cells compared to 16/10⁶ in the negative

control and $966/10^6$ in the positive control (200 µg/ml EMS). Relative survival ranged from 90-27% compared to 55% in the controls

165. Exposure to PCP has been shown to have a genotoxic effect on the epithelial cells lining the human nasal mucosa using the comet assay (Tisch *et al.*, 2005). The cells were isolated from nasal mucosae which were removed during surgical treatment of chronic nasal conditions and were incubated with 0.3-1.2 mmol PCP. There was a lower response in the cells of the inferior nasal concha compared to the middle nasal concha; the reason for this was unclear but it was suggested it could reflect differences in metabolism.

In vivo genotoxicity studies

166. In a mouse spot test, pregnant mice were injected intraperitoneally with 50 or 100 mg purified PCP/kg body weight on day 10 of gestation. Examination of offspring revealed an increased frequency of coat spots indicative of a weak mutagenic response (Fahrig *et al.*, 1978). This was estimated to be 0.6 and 0.7 % in the 50 mg/kg groups and 0.6% in the 100 mg/kg group versus 0.1% in controls), indicative of a weak mutagenic response (Fahrig *et al.*, 1978). The calculation of frequency included spots considered to be “of genetic relevance.”
167. Chromosome analyses were carried out on peripheral lymphocytes from 22 male workers at a factory producing PCP (Bauchinger *et al.*, 1982). All of the workers were smokers and were compared to controls of which 9/22 smoked. A small but significant increase in the frequency of dicentric and acentric chromosomes was observed compared to smoking controls but there was no difference in the frequency of sister-chromatid exchange in smoking PCP workers compared to smoking controls. This paper is attached at Annex 1.

Other studies

168. McConnell *et al.* (1991) looked at the effects of two grades of PCP in a 2 year feeding study in B6C3F1 mice. Diets containing 100 or 200 ppm of either a technical grade PCP composite or 100, 200 or 600 ppm Dowicide EC-7 (a commercial grade of PCP with lower levels of contaminants) were fed to groups of 50 male and 50 female mice for 2 years. Control groups consisted of 35 animals. The average daily doses of technical-grade PCP and Dowicide EC-7 were approximately 0, 17-18, 35 and 114-118 mg/kg respectively. Survival did not appear to be significantly affected by exposure to either type of PCP at the doses used. Dose related increases in hepatocellular adenomas and carcinomas were observed in the male and female mice exposed to both the technical grade PCP and the EC-7 but the increase was less marked in females exposed to the technical PCP. Pheochromocytomas of the adrenal

gland in exposed male mice were significantly increased compared to the controls for both the technical grade PCP and the EC-7. These neoplasms were also increased in female mice exposed to the EC-7 only.

Haemangiosarcomas of the spleen and liver were increased in females receiving both the technical grade PCP and the EC-7.

169. The impurities detected in the technical grade PCP were hexachlorodibenzodioxin, heptachlorodibenzodioxin, octachlorodibenzodioxin, pentachlorodibenzofuran, hexachlorodibenzofuran, heptachlorodibenzofuran, octachlorodibenzofuran, heptachlorohydroxydiphenyl ether, octachlorohydroxydiphenyl ether, nonachlorohydroxydiphenyl ether, hexachlorohydroxydibenzofuran and heptachlorohydroxydibenzofuran. In the EC-7, higher levels of hexachlorobenzene were present, but the other impurities detected, hexachlorodibenzodioxin, heptachlorodibenzodioxin, octachlorodibenzodioxin, hexachlorodibenzofuran and heptachlorodibenzofuran, were present at much lower levels. It was concluded that both grades of PCP were carcinogenic to mice but taking into account the dose-responses and the findings of other NTP studies, it was considered that the responses appeared to be due almost exclusively to the PCP itself, but that there could be a minimal potentiating influence by the contaminants in the induction of liver neoplasms in male mice.

PCP summary/conclusions

170. PCP is not mutagenic in bacterial cells. There is insufficient information to assess genotoxicity in mammalian cells *in vitro* or to assess *in vivo* genotoxicity.

Chloroanisoles

171. Few data have been identified on the genotoxicity of chloroanisole compounds, with the exception of pentachloroanisole.

In vitro genotoxicity

Bacterial cells

172. Concentrations of 10-1000µg/ml pentachloroanisole were negative for mutation in *Salmonella* strains TA 100 or TA 1535 in the absence or presence of S9 (rat or hamster) (NTP, 1993). Positive responses were obtained in the absence of S9 in strains TA 1537 and 98. The number of revertants in TA 1537 was 7, 6, 6, 7, 15 and 25 at concentrations of 0, 100, 333, 1000 and 10,000 µg/plate compared to 124 in the positive control for trial 1 and 4, 5, 5,

12, 19 and 34 at concentrations of 0.333, 1000, 6666 and 10,000 µg/plate compared to 254 in the positive control for trial 2.

Mammalian cells

173. Pentachloroanisole was positive in the mouse lymphoma assay in the presence but not absence of S9 (McGregor *et al*, 1987). In the absence of S9, average mutant fractions of 28, 28, 50, 51 and 42 were observed at concentrations of 0, 62.5, 125, 250 and 500 µg/ml compared to 145 for 250 µg/ml EMS. In a second run of the experiment, average mutant fractions of 52, 43, 52, 63, 71 and 83 were observed at concentrations of 0, 31.25, 62.5, 125, 250 and 500 µg/ml compared to 244 for 250 µg/ml EMS. Relative total growth ranged from approximately 100% to 18% over the concentration range. In the presence of S9 activation, 4 trials were conducted over different concentration ranges. The results were as below

Trial 1

Concn µg/ml	0	31.25	62.5	125	250	500	3 M-C
Average MF	71	<u>226</u>	<u>113</u>	102	<u>138</u>	<u>127</u>	<u>741</u>

Trials 2&3

Concn µg/ml	0	50	75	100	125	150	3 M-C
Average MF	26	<u>77</u>	<u>56</u>	<u>54</u>	<u>59</u>	<u>57</u>	<u>374</u>
	28	<u>49</u>	<u>53</u>	45	45	40	<u>172</u>

Trial 4

Concn µg/ml	0	15	25	50	75	3 M-C
Average MF	78	72	86	<u>145</u>	<u>324</u>	<u>491</u>

NB. Results are positive where underlined

Positive results did not occur until relative total growth was 40% or lower. The LOED calculated for the trial was 31µg/ml in one and 50 µg/ml in the others.

174. Pentachloroanisole induced SCEs but not chromosome aberrations with and without S9 (NTP, 1993). A delayed harvest protocol was used in the SCE assay to offset the cell cycle delay induced by pentachloroanisole. At doses of 75, 100 and 125 µg/ml pentachloroanisole in the absence of S9, the relative % of SCEs per cell were -3.5, 27.82 and 21.73% respectively compared to 29.29 and 236.5% in the positive control, 0.001 and 0.01µg/ml

mitomycin C. In trial 1, in the presence of S9, concentrations of 117, 350 and 700 µg/ml pentachloroanisole, the relative % of SCEs per cell were 5.57, 11.00 and 65.82% respectively compared to 37.37 and 227.04% in the positive control, 0.35 and 2.0 µg/ml cyclophosphamide. In trial 2, in the presence of S9, the relative % of SCEs per cell were 20.43, 54.00 and 39.42% at concentrations of 595, 648 and 700 µg/ml respectively compared to 35.24 and 204.13% in the positive controls.

Other studies

175. In a 2 year study in F344 rats, groups of 70 male and female rats were given 0, 10, 20 and 40 mg/kg pentachloroanisole by gavage for five days per week (NTP, 1993). It was concluded that there was some evidence of carcinogenicity in male rats, on the basis of an increase in benign pheochromocytomas in the adrenal medulla. There was equivocal evidence in female rats based on a marginally increased incidence of benign pheochromocytomas in the adrenal medulla. A bioassay was also conducted in groups of 70 male and female B6C3F1 mice who were treated with 0, 10, 20 and 40 mg/kg pentachloroanisole by gavage five/seven days per week. It was concluded that there was some evidence of carcinogenicity in male mice, on the basis of an increase in benign pheochromocytomas in the adrenal medulla and hemangiosarcomas of the liver. There was no evidence of carcinogenicity in female mice.

Pentachloroanisole summary/conclusion

176. There is some evidence that pentachloroanisole has mutagenic effects in bacterial and mammalian cells *in vitro* and clastogenic effects *in vitro*. There are no data to assess *in vivo* genotoxicity.

QSAR

177. QSAR data will be provided separately.

Summary and discussion

178. Chlorophenols are organic chemicals formed from phenol; nineteen congeners are possible, ranging from monochlorophenols to the fully chlorinated pentachlorophenol. Chlorophenols, particularly trichlorophenols, tetrachlorophenols, and pentachlorophenol, also occur as sodium or potassium salts. Chlorophenols were widely used in the wood preservation industry and are intermediates in pesticide production. Industrially produced chlorophenols

may be contaminated with compounds such as polychlorinated dibenzodioxins and polychlorinated dibenzofurans. The effects of impurities on the result of the genotoxicity studies is uncertain; while dioxin contamination might affect the results of *in vivo* bioassays for promotion and carcinogenicity rather than *in vitro* genotoxicity studies, positive results attributable to impurities rather than chlorophenols could occur. Information on the source of test chemicals is attached in Annex 2.

179. Low levels of chlorophenols can be found in wine; these occur due to the action of naturally occurring airborne fungi on contaminants in cork; though low levels may also occur naturally in wine.
180. Chlorophenols are rapidly absorbed following oral administration and do not appear to accumulate. Depending on the compound, oxidation and/or dechlorination can occur, with a variety of metabolites being formed which can include chlorocatechols, quinones, hydroxyquinones and dichloromethoxy phenols. Chlorophenols and their metabolites tend to be conjugated with glucuronic acid or sulphate prior to excretion.
181. In general chlorophenols increase in acute systemic toxicity with increasing chlorination.
182. The available genotoxicity data for the chlorophenols are summarised in Tables 1 to 3. In general, the monochlorophenols appear to be negative in bacteria, with the exception of one study (Strobel and Grummt, 1987) where some positive results were reported in contrast to the results of other authors. In mammalian cell systems assessing mutation the monochlorophenols are negative, but limited data in mammalian cells suggest some clastogenic activity. The dichlorophenols are generally negative in the Ames test but some are weakly positive in the *E.coli* prophage assay, they are also generally negative in mammalian cell systems but limited data suggest some clastogenic activity. The trichlorophenols are generally negative in the Ames test but some test positive in the *E.coli* prophage assay, they are also generally negative in mammalian cell systems but with some data suggesting clastogenic activity. There are far fewer data available for tetrachlorophenols and PCP than the other chlorophenols but they are generally negative in the Ames test and mammalian cell systems where available, but some test positive in the *E.coli* prophage assay. 2,4,6-TCP has been reported to cause DNA strand breaks and PCP metabolites have been reported to bind to both DNA and protein *in vitro*.

Table 1. *In vitro* genotoxicity studies on chlorophenol isomers – bacterial and yeast systems

Isomer	Ames	<i>E coli</i> prophage	<i>B subtilis</i> rec assay	<i>umu</i>	<i>S.cerevisiae</i>	References
2-chlorophenol	-ve in TA98,100,1535,1537 + and – S9 -ve in TA 100– S9	-ve + and – S9		-ve + and – S9		Haworth <i>et al</i> , 1983; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990 Ono <i>et al</i> , 1992,
3-chlorophenol	-ve in TA100, 98, 1535,1537 + and – S9 -ve in TA 100 – S9 -ve in TA 98 + and –S9 +ve TA 100 –S9, TA97 + and –S9, TA104 +S9	-ve + S9 equivocal -S9				Haworth <i>et al</i> , 1983; Rapson <i>et al</i> , 1980; Strobel and Grummt, 1987; DeMarini <i>et al</i> , 1990
4-chlorophenol	-ve in TA98, 100,1535,1537 + and – S9 -ve in TA 100 – S9 -ve in TA 98, 100, 1535, 1538 marginal +ve TA1537 -S9 +ve TA98,100, 97, 104 +S9 All –ve –S9.	-ve + and – S9		-ve + and - S9		Haworth <i>et al</i> , 1983; Rapson <i>et al</i> , 1980; Seufferer <i>et al</i> , 1979; Strobel and Grummt, 1987; DeMarini <i>et al</i> , 1990; Sakagami <i>et al</i> , 1988.
2,3-dichlorophenol	-ve in TA98, 100,1535,1537 + and – S9 -ve in TA98, 100,1535,1537 + and – S9 -ve in TA 100 – S9	weak +ve +S9, -ve -S9				Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
2,4-dichlorophenol	-ve in TA98,100,1535,1537+ and – S9 -ve in TA98, 100,1535,1537, + and – S9 -ve in TA 100– S9 -ve in TA98,100,1537 + and – S9 Equivocal TA 1535+ hamster S9 -ve in G46, TA1535,TA 1000, C3076, TA 1537, D3052, TA 1538, TA 98 + and –S9	-ve + and –S9 -ve + and –S9		-ve +S9, +ve –S9		Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Rapson <i>et al</i> , 1980;NTP, 1989; Probst <i>et al</i> , 1981 DeMarini <i>et al</i> , 1990 Ono <i>et al</i> ; 1992.
2,5-dichlorophenol	-ve in TA98, 100,1535,1537 + and – S9	weak +ve				Haworth <i>et al</i> , 1983; Räsänen <i>et</i>

Isomer	Ames	<i>E coli</i> prophage	<i>B subtilis</i> rec assay	<i>umu</i>	<i>S.cerevisiae</i>	References
	-ve in TA98,100,1535,1537, + and – S9 -ve in TA100 –S9	+S9,-ve -S9				<i>al</i> , 1977; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
2,6-dichlorophenol	-ve in TA100,1535,1537,1538 + and – S9 -ve in TA98, 100,1535,1537 + and – S9 -ve in TA100,1535,1537,1538, TA1358 + and – S9 -ve in TA100 –S9	weak +ve+S9,-ve -S9				Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Nestmann <i>et al</i> , 1980; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
3,4-dichlorophenol	-ve in TA100,1535,1537,1538 + and – S9 -ve in TA98, 100,1535,1537 + and – S9 -ve in TA100 –S9	-ve + and -S9				Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
3,5-dichlorophenol	-ve in TA100,1535,1537,1538 + and – S9 -ve in TA98, 100,1535,1537 + and – S9 -ve in TA100 –S9	weak +ve+S9,-ve -S9				Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
2,3,4-trichlorophenol	-ve in TA100 –S9	+ve +S9, weak +ve-S9				Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
2,3,5-trichlorophenol	-ve in TA98,100,1535,1537 + and – S9 -ve in TA100 –S9	Equivocal +S9,-ve -S9				Räsänen <i>et al</i> , 1977; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990
2,3,6-trichlorophenol	-ve in TA98,100,1535,1537 + and – S9 “-ve” no further data -ve in TA 97, 100, 104 + and –S9, +ve in TA98 +S9, -ve –S9.	+ve +S9, -ve- S9				Räsänen <i>et al</i> , 1977; NTP, 1987; Strobel and Grummt, 1987; DeMarini <i>et al</i> , 1990
2,4,5-trichlorophenol	-ve TA98,100, 102, 104 + and – S9 -ve TA 98,100, 1535, 1537 + and – S9 -ve TA 98,100, 1535, 1537 + and – S9 -ve TA 98,100, 1535, 1537, 1358 + and – S9	+ve +S9, weak +ve-S9 +ve +S9, -ve- S9		-ve + or – S9		George <i>et al</i> , 1992; Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Nestmann <i>et al</i> , 1980; Strobel and Grummt, 1987; DeMarini <i>et al</i> , 1990; Ono <i>et al</i> , 1992.

Isomer	Ames	<i>E coli</i> prophage	<i>B subtilis</i> rec assay	<i>umu</i>	<i>S.cerevisiae</i>	References
	-ve TA97 + and -S9,+ve TA100-S9, -ve -S9 -ve TA 98, 104 + and -S9					
2,4,6-trichlorophenol	-ve TA 98,100, 1535, 1537 + and - S9 -ve TA 98,100, 1535, 1537 + and - S9 -ve TA 98,100, 1537 + and - S9 -ve in TA100 -S9 +ve TA 97,98, 104 + S9, -ve TA 97,98, 104 -S9 -ve in TA100 + and -S9	+ve	+ve+ S9, wek +ve, - S9		Weak +ve forward mutation -ve re-combination	Haworth <i>et al</i> , 1983; Räsänen <i>et al</i> , 1977; Kinae <i>et al</i> , 1981; Rapson <i>et al</i> , 1980; DeMarini <i>et al</i> , 1990; Fahrig <i>et al</i> , 1978;
3,4,5-trichlorophenol	-ve for mutagenicity- no further data					NTP, 1987
2,3,4,5-tetrachlorophenol	-ve TA 97, 98,100, 1535 + and - S9	+ve + s9, -ve-s9				Zeiger <i>et al</i> , 1988; DeMarini <i>et al</i> , 1990
2,3,4,6-tetrachlorophenol	-ve TA98, 100, 1535, 1537 + and -S9 -ve TA97, 98, 100, 1535, + and -S9	+ve + S9, -ve -S9		+ve + and - S9		Räsänen <i>et al</i> , 1977; Zeiger <i>et al</i> , 1988; DeMarini <i>et al</i> , 1990; Ono <i>et al</i> , 1992.
2,3,5,6-tetrachlorophenol	-ve TA97, 98, 100, 1535, + and -S9	weak +ve - S9.		+ve + and - S9		Zeiger <i>et al</i> , 1988; DeMarini <i>et al</i> , 1990
pentachlorophenol	-ve TA98, 100, 1535, 1537 + and -S9	+ve, +S9, weak +ve -S9		+ve		Haworth <i>et al</i> , 1983; DeMarini <i>et al</i> , 1990; Ono <i>et al</i> , 1992.

Table 2. *In vitro* genotoxicity studies on chlorophenol isomers – mammalian cells

Isomer	L5178Y	HPRT/TG resistance	Cell transformation	UDS	Chromosome aberrations	SCE	c-mitosis, aneuploidy, diploidy	Comet	References
2-chlorophenol							Slight-moderate increase in c-mitosis, aneuploidy . (CH V79 cells) -S9?		Önfelt, 1987.
3-chlorophenol									
4-chlorophenol			-ve (SHE cells)	-ve (SHE cells)	+ve (SHE cells) + S9			-ve (mouse lymphoma and human fibroblasts)	Hagiwara <i>et al</i> , 2006; Hamaguchi <i>et al</i> , 2000; Ribeiro <i>et al</i> , 2004
2,3-dichlorophenol									
2,4-dichlorophenol	+ve, -S9	-ve (CH V79 cells) – S9		-ve (rat hepatocytes)	-ve (CHO cells) + and – S9	+ve (CHO cells) + and – S9	+ve moderate increase in aneuploidy, polyploidy, -S9 (CH V79 cells)		NTP, 1989; Jansson and Jansson, 1986; Probst <i>et al</i> , 1981; Önfelt, 1987.
2,5-dichlorophenol		-ve (CHO cells) + and – S9							Tegethoff <i>et al</i> , 2000
2,6-dichlorophenol		-ve CH V79 cells – S9 -ve CH V79 cells + and – hepatocyte activation							Jansson and Jansson, 1986; Hattula and Knuutinen, 1985

Isomer	L5178Y	HPRT/TG resistance	Cell transformation	UDS	Chromosome aberrations	SCE	c-mitosis, aneuploidy , diploidy	Comet	References
3,4-dichlorophenol									
3,5-dichlorophenol							-ve, -S9		Önfelt, 1987.
2,3,4-trichlorophenol									
2,3,5-trichlorophenol									
2,3,6-trichlorophenol					+ve (CHO cells, CH V79 cells)+ and - S9 +ve (CHO cells)	-ve (CHO cells)			Armstrong <i>et al</i> , 1993; NTP, 1987.
2,4,5-trichlorophenol		-ve, -S9			+ve (CHO cells, CH V79 cells) + and -S9				Jansson and Jansson, 1986; Armstrong <i>et al</i> , 1993;
2,4,6-trichlorophenol	+ve, -S9	-ve, -S9 weak +ve, -S9			-ve (CHO cells) + and -S9 +ve (CHO and CH V79 cells) Small ↑micronuclei (CH V 79 cells - S9)	-ve (CHO cells) + and -S9	Small↑ Hyperdiploidy (CH V79 cells)		McGregor <i>et al</i> , 1988; Jansson and Jansson, 1986; Jansson and Jansson, 1992;Hattula and Knuutinen, 1985; Galloway <i>et al</i> (1987)
3,4,5-trichlorophenol					-ve (CHO cells)	-ve (CHO cells)	Slight to moderate increase in c-mitosis, aneuploidy , -S9		NTP, 1987; Önfelt, 1987.
2,3,4,5 tetrachlorophenol									
2,3,4,6-tetrachlorophenol		Weak +ve CH V79 cells (direct)							Hattula and Knuutinen, 1985; Jansson and Jansson, 1986;

Isomer	L5178Y	HPRT/TG resistance	Cell transformation	UDS	Chromosome aberrations	SCE	c-mitosis, aneuploidy , diploidy	Comet	References
		-ve CHV79 cells with hepatocyte co-culture. -ve CH V79 cells -S9							
2,3,5,6-tetrachlorophenol									
pentachlorophenol		-ve CHV79 cells-S9						+ve isolated nasal cells	Jansson and Jansson, 1986;Tisch <i>et al</i> ; 2005

Table 3. *In vivo* genotoxicity studies on chlorophenol isomers

Isomer	Drosophila	Micronuclei	SCE	Chromosome aberrations	Mouse spot test	Alkaline elution	Reference
2-chlorophenol			Up to 69 mg/kg bw by stomach tube, -ve in testes and bone marrow of mice				Borzecella <i>et al</i> , 1985;
3-chlorophenol							
4-chlorophenol							
2,3-dichlorophenol			-ve result cited for i.p. admin				Borzecella <i>et al</i> , 1985;
2,4-dichlorophenol			-ve in testes and bone marrow in mice, 50-5000 mg/kg bw via drinking water				Borzecella <i>et al</i> , 1985;
2,5-dichlorophenol		-ve in bone marrow in mice 1500 mg/kg bw by gavage					Tegethoff <i>et al</i> , 2000
2,6-dichlorophenol		+ve in bone marrow in rats. No further details.					Chung, 1978
3,4-dichlorophenol							
3,5-dichlorophenol							
2,3,4-trichlorophenol							
2,3,5-trichlorophenol							
2,3,6-trichlorophenol							
2,4,5 –						-ve liver and WBCs in rats	Kitchin and Brown,

Isomer	Drosophila	Micronuclei	SCE	Chromosome aberrations	Mouse spot test	Alkaline elution	Reference
trichlorophenol						given 164 mg/kg bw orally	1988
2,4,6-trichlorophenol	-ve, fed in sucrose solution				Weak +ve, 50-100 mg/kg bw i.p. in mice	-ve liver and WBCs, 2 oral doses used.	Valencia <i>et al</i> , 1985; 1959; Fahrig <i>et al</i> , 1978; Kitchin and Brown, 1988
3,4,5-trichlorophenol							
2,3,4,5-tetrachlorophenol							
2,3,4,6-tetrachlorophenol						-ve liver and WBCs in rats given 193 mg/kg	Kitchin and Brown, 1988
2,3,5,6-tetrachlorophenol							
pentachlorophenol			-ve in lymphocytes of PCP workers	small↑ in dicentric and acentric chromosomes in lymphocytes of PCP workers	Weak +ve, 50-100 mg/kg bw i.p. in mice		Bauchinger <i>et al</i> , 1982; Fahrig <i>et al</i> , 1978;

183. In general, the positive results reported for chlorophenol compounds *in vitro* are clastogenic effects occurring in mammalian cells; possible spindle damage has also been reported. However, prophage induction and results reported for the *umu* assay (the latter largely as a secondary citation) indicate induction of SOS repair in bacterial cells and thus the occurrence of DNA damage in these systems; this is more apparent for the higher chlorophenols. Metabolic data suggest that some of the chlorophenols, notably the TCPs, TECPs and PCP are metabolised to chlorohydroquinones and hydroquinones; there are no data available to indicate whether MCPs and DCPs can also form hydroquinones but it seems likely that this could occur. Quinones are able to undergo redox cycling, forming semiquinones and thus may cause damage through the formation of reactive oxygen species. Data from *in vitro* studies suggest that certain chlorophenols can bind to DNA and protein *in vitro* and can also induce DNA strand breaks, the chlorophenol binding to protein was largely mediated by reactive oxygen species, and while this was not assessed in the DNA binding experiments, the CP concerned (PCP) was readily metabolised to hydroquinone metabolites in a comparable enzyme system.
184. *In vivo* studies of chlorophenols, where available, are generally negative. The chlorophenols may have some promoting activity. In conventional animal bioassays 2,4 DCP was not carcinogenic in rats or mice whereas 2,4,6-TCP was carcinogenic in male rats and mice of both sexes. In the latter study, there were 17 minor contaminants and dioxin was not measured, so it is uncertain whether contamination could have explained the findings of this assay.
185. Studies of workers at a PCP manufacturing site suggested a small increase in chromosome aberrations, but not sister chromatid exchange, compared to controls; though it is unclear what else the workers may have been exposed to.
186. It is possible that the genotoxic effects associated with certain chlorophenols may be due to reactive oxygen species. However, it is unclear whether this would occur *in vivo*. The data available are limited.

Questions for the COM

187. Members are asked to consider the available evidence relating to genotoxicity of the chlorophenols and to answer the following questions:

- i) Should the chlorophenol congeners be evaluated individually, as groups of mono-, di-, tri-, tetra- and pentachlorophenols, or as an overall class of chlorophenols?
- ii) Can members comment on the each chlorophenol or group of chlorophenols in turn?
- iii) Do the available data allow conclusions to be reached on genotoxic modes of action for the chlorophenol congeners or classes?
- iv) Does the available information indicate that the modes of action are likely to exhibit a threshold, or that it would be prudent to assume that the chlorophenols are non-threshold genotoxic substances in the absence of further information?
- v) Can any conclusions be drawn with respect to chloroanisoles?

Food Standards Agency

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COMMITTEE ON THE MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT

REVIEW OF GENOTOXICITY OF CHLOROPHENOLS

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Secretariat

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Annex 2 - MUT/2011/13

COMMITTEE ON THE MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT

REVIEW OF GENOTOXICITY OF CHLOROPHENOLS

Information on chemical purity for experimental systems.

Reference	Congener (s)	Purity/source	Comment
Bacteria and yeast			
Haworth <i>et al</i> , 1983;	2-CP 3-CP 4-CP 2,3-DCP 2,4-DCP 2,5-DCP 2,6-DCP 3,4- DCP 3,5-DCP 2,4,5-TCP 2,4,6-TCP PCP	Commercial- 98% purity Commercial- Reagent Commercial- Practical Commercial- 98% purity Commercial- 99% purity Commercial- 98% purity Commercial- 99% purity Commercial- 99% purity Commercial- 99% purity - Practical Commercial- 96% purity	As stated on label. No information on possible contaminants provided.
Rapson <i>et al</i> , 1980;	2-CP 3-CP 4-CP 2,3-DCP 2,4-DCP 2,5-DCP 2,6-DCP 3,4- DCP 3,5-DCP 2,3,4-TCP 2,3,5-TCP 2,4,6-TCP	Not stated.	
DeMarini <i>et al</i> , 1990	2-CP 3-CP 4-CP 2,3-DCP 2,4-DCP 2,5-DCP 2,6-DCP 3,4- DCP 3,5-DCP 2,3,4-TCP 2,3,5-TCP 2,3,6-TCP 2,4,5-TCP 2,4,6-TCP	Obtained from NTP Reagent Grade 98% purity Practical Grade 98% purity 99% purity 98% purity 99% purity 99% purity 99% purity 97% purity 99% purity 99% purity 99% purity Practical Grade	

	3,4,5-TCP 2,3,4,5- TeCP 2,3,4,6- TeCP 2,3,5,6- TeCP PCP	Unknown 98% purity Unknown 99% purity 92% purity	
Ono <i>et al</i> ; 1992,			Secondary reference
Strobel and Grummt, 1987	2-CP 3-CP 4-CP 2,3,6-TCP 2,4,5-TCP 2,4,6-TCP	Commercially obtained, no purity information	
Seuferer <i>et al</i> , 1979	4-CP	Commercially obtained, no purity information	
Räsänen <i>et al</i> , 1977;	2,3-DCP 2,4-DCP 2,5-DCP 2,6-DCP 3,4- DCP 3,5-DCP 2,3,5-TCP 2,3,6-TCP 2,4,5-TCP 2,4,6-TCP 2,3,4,6- TeCP	Commercially obtained, no purity information	
Probst <i>et al</i> , 1981	2,4-DCP	Commercially obtained, no purity information	
Nestmann <i>et al</i> , 1980;	2,6-DCP	Commercially obtained, no purity information	
NTP, 1989	2,4-DCP	Commercially obtained, 99% pure. Dioxins and furans and hexachlorobenzene analysed for but not detected. Traces of 1,4 and 2,6 DCP detected.	
George <i>et al</i> , 1992	2,4,5-TCP	Commercially obtained, no purity information	
Kinae <i>et al</i> , 1981;	2,4,6-TCP		Secondary citation only
Zeiger <i>et al</i> , 1988			
Mammalian cells			
Önfelt, 1987.	2-CP 2,4-DCP 3,5-DCP 3,4,5-TCP	Provided, purified, from non- commercial source	
Hagiwara <i>et al</i> , 2006	4-CP	Commercially obtained, no purity information	
Hamaguchi <i>et al</i> , 2000	4-CP	Commercially obtained, >95% pure	
Ribeiro <i>et al</i> , 2004	4-CP	Commercially obtained, no purity information	
Jansson and Jansson, 1986	2,4-DCP 2,6-DCP 2,4,5-TCP 2,4,6-TCP	Commercially obtained, >99.5% pure. No dioxins detected.	

	2,3,4,6- TeCP		
Tegethoff <i>et al</i> , 2000	2,4-DCP	Commercially obtained, >98% pure	
Hattula and Knuutinen, 1985	2,6-DCP 2,4,6-TCP 2,3,4,6- TeCP PCP	Commercially obtained and further purified to >99.95% pure	
Armstrong <i>et al</i> , 1993;	2,3,6-TCP 2,4,5-TCP 2,4,6-TCP	Commercially obtained and > 99% pure , sample of 2,4,6-TCP further purified to >99.95% pure	
Fahrig <i>et al</i> , 1978	2,4,6-TCP PCP	Commercially obtained and further purified to >99% pure	
McGregor <i>et al</i> , 1988	2,4,6-TCP	Supplied by NTP no further purity data provided	
Galloway <i>et al</i> (1987)	2,4,6-TCP	Supplied by NTP no further purity data provided	
Tisch <i>et al</i> ; 2005	PCP	Commercially obtained >99% pure	
<i>In vivo studies</i>			
Borzecella <i>et al</i> , 1985	2-CP 2,4-DCP	Source not stated	Conference proceedings
Boutwell and Bosch, 1959	2-CP 3-CP 2,4,5-TCP 2,4,6-TCP	Not stated.	
Exon and Koller, 1981	2-CP 2,4-DCP 2,4,6-TCP	Commercially obtained, 98-99% pure	
Tegethoff <i>et al</i> , 2000	2,5-DCP	Commercially obtained, >98% pure	
Chung, 1978	2,6 DCP		Secondary reference
Kitchin and Brown, 1988	2,4,5-TCP 2,4,6-TCP 2,3,4,6- TeCP		Abstract only
Valencia <i>et al</i> , 1985;	2,4,6 TCP		Secondary reference
Fahrig <i>et al</i> , 1978;		Commercially obtained and further purified to >99% pure	
Bauchinger <i>et al</i> 1982	PCP	Industrial exposure	Could be multiple contaminants, many participants smoked