COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Food Surveillance Information Sheet: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food

Issue

1. The Committee is asked to comment on the risks associated with polychlorinated naphthalenes and chlorinated paraffins in food following an investigation of these compounds in a number of foodstuffs.

Introduction

2. The Food Standards Agency has recently completed an investigation of polychlorinated naphthalenes and chlorinated paraffins in food. An unpublished draft of this work is attached at Annex A.

3. The available literature for the two groups of compounds are summarised in this paper. In the case of the chlorinated paraffins, the available data were recently reviewed by the UK Health and Safety Executive (HSE) as rapporteur of the risk assessments under the EU Existing Substances Regulation. Only papers published after these risk assessments in 1999 for short chain compounds and 2004 for medium chain compounds have been included in this document. The HSE reviews are attached at Annexes B and C. The search process used to identify the papers is presented in Annex D.

Background

4. Polychlorinated naphthalenes (PCNs) are a group of 75 chemicals collectively referred to as congeners, and they have structures similar to those of dioxins. They were formerly manufactured extensively, and they possess high chemical and thermal stability, good weather resistance, good electrical insulating properties and low flammability properties. As a result, they were commonly used in electrical equipment such as switchgear, sometimes as a replacement for polychlorinated biphenyls. They were sold commercially as Halowax formulations. This usage is now banned in most countries, including the UK. PCNs can also be produced as combustion products (IPCS, 2001).

5. Chlorinated paraffins (CPs) are a much larger group of chemicals and comprise several thousand individual chemicals. They are chlorinated linear hydrocarbons with between 10 and 30 carbon atoms. Structurally these chemicals bear little resemblance to dioxins. They are manufactured by the chlorination of liquid

paraffin. They contain varying numbers of chlorine atoms, with a maximum of one chlorine atom per carbon. Depending on the length of the carbon skeleton CPs are classified as short (SCCPs: C10-13), medium (MCCPs: C14-17) and long chain (LCCPs: C18-26). The industrial applications of CPs vary depending on the chain length, ranging from industrial lubricants in metal manufacturing to as additives in plastics, paints and sealants. A common current use is as flame retardants (UK HSE, 1999; UK HSE, 2004).

6. PCNs have been detected in fish and human milk in other countries, while CPs have occasionally been detected in fish. This investigation was carried out as there are currently no UK data for either group of compounds.

Polychlorinated Naphthalenes

Chemistry

7. Theoretically, there are 75 possible PCN congeners containing between one and eight chlorine atoms bound to the naphthalene structure shown in Figure 1 (IPCS, 2001).

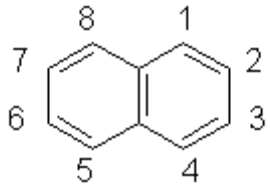

Figure 1: Naphthalene structure and the positions where chlorine atoms can potentially bind (taken from IPCS, 2001)

8. In a review of chlorinated naphthalenes it was determined that persistence of PCNs decreases with decreasing chlorine content and depends on the number of vicinal carbon atoms unsubstituted with chlorine (Falandysz, 2003).

Kinetics

Absorption

9. Four male Wistar rats were given a single oral dose of radiolabelled 1,2-dichloronaphthalene and serial blood samples were taken from the jugular vein hourly for 8 hours and then 24 and 48 hours after administration. At 48 hours, animals were sacrificed and tissue distribution was investigated. The results showed that rapid absorption occurs with the highest levels occurring in the sample taken at 1 hour. Levels gradually declined over the first 8 hours. By 48 hours, radioactivity levels in blood were approximately 20% of the peak 1 hour level (Chu *et al.*, 1977a).

Distribution

10. Groups of male JCL:ICR mice were given a 1 mg single intraperitoneal dose of either 1,8-dichloronaphthalene, 2,7-dichloronaphthalene or octachloronaphthalene. After 3 or 6 hours, 1, 3, 7, 14, or 28 days groups of 3 animals were sacrificed and organs were collected to analyse the distribution of the compounds. After the first day, adipose tissue had the highest percentage of the total dose for all three compounds and heart and brain had the lowest. The relative distribution in other tissues did not show a fixed relationship. For all 3 PCNs the maximum tissue concentration was reached within 3 hours of dosing in all tissues except octachloronaphthalene in adipose tissue which increased gradually up to day 7. The biological half life for all three compounds was longest in the adipose tissue, which the authors suggested to be due to the lipophilic properties of PCNs. It was also notable that the half-life for 2,7-dichloronaphthalene was longer than 1,8-dichloronaphthalene in almost all tissues suggesting that the position of the substituted chlorine has an effect. In addition octachloronaphthalene had a longer biological half-life than the two dichloro- congeners suggesting that the number of chlorine atoms also has an influence (Oishi and Oishi, 1983).

11. Ten male Wistar rats were given a single oral gavage dose of radiolabelled 1,2-dichloronaphthalene and placed in metabolic cages. 5 animals were sacrificed after 24 hours for tissue distribution studies and the remainder kept for 7 days before the residual tissue concentration was determined. Animals used in the absorption study described above provided 48 hour tissue distribution samples. After the first day, the liver and intestines had the highest percentage of the total dose however by day 7, radioactivity could only be detected in the skin and adipose tissue and corresponded to 0.05% of the total dose (Chu *et al.*, 1977a).

12. A study where a single dose of 10 mg/kg b.w. tritiated 1,2,3,4-tetra- and 1,2,3,4,5-pentachloronaphthalene was administered to male Outbred Wist rats by intraperitoneal injection showed that the highest levels were found in fat tissues, liver, kidney and adrenals. The highest levels in plasma and tissues were reached after 12 hours for the tetra- and 24 hours for the penta- congener (Kilanowicz *et al.*, 2004).

13. Technical mixtures of PCNs have been used to study bioaccumulation. Female Sprague Dawley rats were given a single oral dose of 20 mg/kg b.w. Halowax 1014, which contains tetra- to octa- congeners. Animals were killed after 1, 10, 30 or 120 days and the liver and intraperitoneal fat removed for analysis. No signs of toxicity were observed and weight gain was similar to that of control animals. On the first day after dosing, the PCN pattern in the samples was similar to that of the technical mixture though the relative levels of hepta- and octa- congeners were lower, which the authors suggest may be explained by less effective absorption. A hexa- congener was dominant in the intraperitoneal fat after 10 days and this continued till day 120 when this was the only peak detected. The concentration on day 120 was approximately 50% of that seen on day 1. The same hexa- congener was dominant in the liver after day 1 and the concentration was higher than that seen in adipose tissue (140 fold after 10 days and 5 fold after 120 days). In the same study one animal was given Halowax 1051, which is a mixture of octa- and hepta-congeners with small amounts hexachloronaphthalene present. This animal was

sacrificed after 30 days. One hexa- and one hepta- congener were identified (Asplund *et al.*, 1986).

14. 1- and 2-chloronaphthalene have been administered to pigs to determine the distribution and metabolism of these compounds. An oil emulsion of the compounds was given by retrocarotid injection to female Yorkshire pigs (1 animal per compound, no control stated). Blood samples were obtained after 10, 20 and 40 minutes and every 40 minutes thereafter. After 6 hours, pigs were sacrificed and total urine, bile, blood and organ samples. Highest levels of the two compounds in blood were measured at 10 minutes and declined after this. After 6 hours the highest concentrations of the two compounds were found in brain and kidney though distribution had occurred to liver, lung, skeletal muscle, psoas and heart. In addition, 2-chloronaphthalene was detected at low levels in fat. Metabolites from both compounds were detected in liver and kidney (Ruzo *et al.*, 1976b).

Metabolism

15. Groups of 5 female Holtzman rats were given diet supplemented with 0.69 ppm vitamin A acetate and containing 5000 ppm 1,4-dichloronaphthalene. Detoxication activity was determined by hexobarbital sleep time. Relative liver weight was significantly increased, as was EPN detoxication and O-demethylase activity while hexobarbital sleep time was significantly reduced (Wagstaff, 1971).

16. Male Wistar rats were given a single oral dose of 3 different dichloronaphthalenes. Urine was collected for two days and then analysed by GC-MS or NMR. Following administration of 1,2-dichloronaphthalene, the glucuronide conjugate of 5,6-dichloro-1,2-dihydroxy-1,2-dihydronaphthalene was found in the urine; 2,7-dichloronaphthalene lead to free and conjugated 7-chloro-2-naphthol being present in the urine and 6-chloro-2-naphthol and 2,6-dichloronaphthol were found in urine following administration of 2,6-dichloronaphthalene. The authors hypothesised that hydroxylation and/or hydroxylation-dechlorination are common metabolic pathways for these compounds (Chu *et al.*, 1977b).

17. Three rabbits were given a single 300 mg/kg dose of 2,6-dichloronaphthalene and urine was collected for a week and the combined samples were analysed for metabolites. Four Sprague Dawley rats were treated similarly following a single 1 g/kg dose. Urine analysis showed unchanged 2,6-dichloronaphthalene, a monochloronaphthol derivative and a dichloronaphthol derivative (Chu *et al.*, 1976).

18. Groups of 3 male albino rabbits were given a 1 g single dose of naphthalene, 1-chloronaphthalene, di-, tetra-, penta-, hepta- or octachloronaphthalene by stomach gavage. 24 hour urine samples were collected for 4 days and levels of creatinine, glucosiduronic acids, phenolic compounds, sulfur partitions and mercapturic acid were measured. The results showed that ingestion of penta-, hepta- and octachloronaphthalene did not increase excretion of the urinary metabolites measured. 45% of the administered tetrachloronaphthalene could be accounted for by these metabolites with 38% excreted as glucuronic acid, 3% as mercapturic acid and 4% as ethereal sulphate.1-chloronaphthalene and dichloronaphthalene showed a similar excretion pattern to that of naphthalene with the majority of the dose (40-70%) excreted as glucuronic acid, 13-19% excreted as mercapturic acid and 6-10%

as ethereal sulphate. In addition, for the animals receiving penta- and heptachloronaphthalene, urine and faeces were analysed for unchanged compound. Small and variable amounts of these chlorinated compounds were isolated, however the total 4 day excretion did not exceed 20% of the 1 g dose (Cornish and Block, 1958).

19. A female dog was placed in a metabolism cage and given 1 g of a mixture of penta- and hexachloronaphthalenes. Urine was analysed for total, inorganic and ethereal sulphur, free and conjugated phenols and inorganic and total chloride. There was a significant increase in the ethereal sulphate excretion for the first two days after administration however there was no change in the neutral sulphur fraction or glucuronic acid excretion. No organic chlorine was found in the urine but there was a significant increase in chloride output for 2 days after chloronaphthalene administration. The hypothesis that an early step in PCN metabolism is liberation of chloride was tested in rats receiving a low chloride diet. Excretion of chloride rose immediately on administration of daily doses of 40 mg of the PCN mixture, and on cessation of dosing, chloride excretion fell due to considerable loss of body weight (Cleary *et al.*, 1938).

20. Pigs were administered 30 mg chloronaphthalenes/kg b.w. by retrocarotid injection in corn oil. After 5 hours animals were sacrificed and urine collected. Urine was analysed by thin-layer chromatography confirmed by mass spectrometry and NMR. The results showed that 1-chloronaphthalene produced 2 metabolites, a monohydroxylated compound, confirmed to be 4-chloro-1-naphthol and trace amounts of a dihydroxy compound. 2-chloronaphthalene was shown to be metabolised to 3-chloro-2-naphthol (Ruzo *et al.*, 1975).

21. A female Yorkshire pig was given a single dose of 1-chloro-4-[^{2}H]-naphthalene by injection into the carotid artery. After 6 hours the pig was sacrificed and total urine and bile used for analysis. The major urinary metabolite was identified as 4-chloronaphthol. A similar approach was used to determine that the major metabolite of 1,2-dichloronaphthalene was 3,4-dichloronaphthol, 1,4-dichloronaphthalene is metabolised to 2,4-dichloronaphthol and 1,2,3,4-tetrachloronaphthalene is metabolised to 5,6,7,8-tetrachloronaphthol and 5,6,7,8-tetrachloro-2-naphthol. No metabolites could be isolated following 1,2,3,4,5,6-hexachloronaphthalene administration (Ruzo *et al.*, 1976a).

22. In the distribution and metabolism study described above, 1- and 2-chloronaphthalene were administered to female Yorkshire pigs. After 160 minutes, 4-chloronaphthol from 1-chloronaphthalene was identified in the blood and after 200 minutes, 3-chloro-2-naphthol from 2-chloronaphthalene was found. At 6 hours, metabolites were found in urine and bile (Ruzo *et al.*, 1976b).

Elimination

23. Two rats, which had their bile ducts cannulated, were given radiolabelled 1,2-dichloronaphthalene by injection in the genital vein. Serial bile samples were collected 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hours after administration and analysed for radioactivity. Urine and faeces are the major routes of excretion and approximately 64% of the total dose was excreted after 48 hours. By day 7, 65% of

the total dose was excreted via the bile however 42% of the original dose had been excreted in the faeces by other rats in this study, which did not have bile duct cannulae. This indicated that some reabsorption from the intestine occurs. Analysis of the faeces indicated that the compound is unchanged whereas in the urine, the radioactivity is found as a glucuronide conjugate of a dihydrodiol (Chu *et al.*, 1977a).

24. A study where a single dose of 10 mg/kg b.w. tritiated 1,2,3,4-tetra- and 1,2,3,4,5-pentachloronaphthalene was administered to male Outbred Wist rats by intraperitoneal injection showed that the main route of excretion was the faeces. After 5 days, 65% of the tetra- congener and 70% of the penta- congener had been excreted in the faeces. The half-lives in plasma were found to be biphasic with a half-life of 13 hours for the tetra- congener and 32 hours for the penta- congener for phase 1 and phase 2 half-lives of approximately 173 hours for both congeners (Kilanowicz *et al.*, 2004).

25. As part of a set of experiments described in more detail below, a cow with a 2 month old bull calf was given 5 g/day octachloronaphthalene for 18 days. The calf was allowed to nurse but was otherwise kept separate and no compound was given directly to the calf. The calf received milk from the dairy herd when it did not receive enough from the dam (reduced milk flow was noted from day 7 and ceased at day 29). Autopsy of the calf on day 50 showed moderate toxicity suggesting that the octachloronapthalene or its metabolites may be excreted in the milk (Sikes *et al.*, 1952).

26. The half-lives of hexachloronaphthalene in rat liver and adipose tissue have been determined as 26 and 41 days. Human blood samples have also been analysed and the half-lives calculated as 1.5-2.4 years (IPCS, 2001).

Toxicity

27. The database on PCNs is large and many studies have been carried out on different congeners and in different species. The responses elicited are dependent on both the species studied and level of chlorination. PCNs were studied following occupational exposure causing cases of chloracne and liver disease in the 1930's and 40's. Subsequently, PCNs were found to be the causative agents of bovine hyperkeratosis and numerous studies were carried out in cattle as a result as well as investigations in sheep and pigs followed this to determine the effects in these species.

28. This section summarises the mammalian and avian studies carried out where chlorinated naphthalenes or technical mixtures of chlorinated naphthalenes were test materials. Studies using dermal application where systemic effects were not studied have not been summarised, however irritation and hyperkeratotic responses have been observed which are in line with the effects seen in cattle (bovine hyperkeratosis) and humans (chloracne) (IPCS, 2001). It should also be noted that authors have commented that consideration of tests using technical mixtures should take into account the possibility of dioxin-like impurities, which may also cause an effect (Falandysz, 2003).

Acute toxicity

29. Groups of 15 male Wistar rats were administered a single gavage dose of 0, 250, 500 or 1000 mg/kg b.w. of a mixture of tetra- (54%), penta- (8%), hexa- (23%) and hepta-chloronaphthalene (14%). 5 animals from each group were necropsied at 24, 72 and 240 hours after administration and blood and whole liver were examined. A loss in body weight was observed in all treated rats compared to controls and at all time points, up to a 30% decrease after 240 hours. Relative liver mass was increased at 72 hours in 500 and 1000 mg/kg b.w. groups. Total CYP levels and CYP1A activity were significantly increased in a non-dose related manner at all time points with the highest values after 72 hours. No significant change in serum ALT activity was detected while a slight significant increase in serum SDH activity was observed in all treated groups after 72 and 240 hours. No significant change in liver glutathione (GSH) levels was detected while malondialdehyde was increased at all time points in the 1000 mg/kg b.w. treated group and at 240 hours in the 500 mg/kg b.w. group. The authors suggest that the mechanism of PCNs' toxic action is probably associated with oxidative stress induction due to the changes in malondialdehyde (Galoch *et al.*, 2006).

30. In the study on metabolism of 1-chloronaphthalene, di-, tetra-, penta-, hepta- and octachloronaphthalenes, the authors reported that all three male albino rabbits receiving a 1 g single gavage dose of hepta- and octachloronaphthalene died within 7 days of ingesting the compound, as did one of the rabbits receiving a 1 g single gavage dose of pentachloronaphthalene. In contrast, animals receiving chloronaphthalenes containing up to 4 chlorine atoms showed no apparent signs of toxicity (Cornish and Block, 1958).

31. Calves (n=1) were given single doses of penta- (22 mg/kg) or hexachloronaphthalenes (11 mg/kg) in gelatin capsules. After 3 days, the calves were listless with lacrimation, excessive salivation and had nasal discharges. The calf receiving hexachloronaphthalene was found dead on day 14 though it was noted that it had had secondary complications as a result of ingestion of this level of hyperkeratosis-producing material, which hastened death. Authors noted that while this was the case, lesions observed were characteristic of chloronaphthalenes. Autopsy showed wartlike proliferations in the mouth, enlarged and mottled liver, enlarged and pale kidneys, ulcers in the abomasum, distended gallbladder and pneumonia in the anterior lobes of both lungs. Microscopic examination showed tubule dilatation in the renal cortex and degeneration of tubule cells, bile duct proliferation and degeneration of liver cells and papillary proliferation of the oral mucosa. The calf receiving pentachloronaphthalene was killed on day 57 and showed thickened skin over the head, neck and sides, fibrosis and bile duct proliferation in the liver, enlarged kidneys with numerous grey areas on the surface extending through the cortex. Microscopic examination showed dilatation of the renal tubules in the cortex, squamous metaplasia of the epididymes and ducts of the parotid gland, bile duct proliferation and fibrosis in the liver and hyperkeratosis of the skin (Bell, 1954).

Repeat dose toxicity

32. Oral dosing of mice for 20 days with 2.5 mg every other day led to considerable excitation compared to the control animals. No skin effects were observed even following exposure to a quartz lamp. Similarly, 3 rabbits were fed 0.4 g/day dry chloronaphthalene for 2 months. They were exposed to a quartz lamp after 1, 1.5 and 2 months dosing to determine if this could elicit any skin effects. Slight erythema on the shaved back and ears was observed but there was little difference compared to the controls. The authors carried out further experiments combining exposure routes to elicit effects. 4 rabbits received dry ground chloronaphthalene orally for up to 2 months and were then subjected to repeated skin application on the shaved side and ear within 1 to 2 months. These rabbits were then exposed to quartz lamp irradiation. Severe oedema and reddening occurred on the ears, which were not observed in rabbits that had not had the initial oral exposure. Further exposure did not increase the dermatitis and the reaction disappeared after several sessions. The same effect was observed when 3 rabbits received subcutaneous injections combined with skin application (Shakahnovskava, 1953).

33. In further experiments, fat, phospholipid and cholesterol content of liver from rats, mice and rabbits given chloronaphthalenes on a chronic basis was examined. Total lipid, cholesterol and neutral fat content was increased and the authors suggested that egestion of fats from the liver via phospholipids was impaired as a result of chloronaphthalene exposure. The increased cholesterol content was likely to favour fatty infiltration of the liver. A supplementation study was carried out in mice where animals were given casein in the diet along with chloronaphthalene to assess whether this is able to restore phospholipid production and fat metabolism. While phospholipid content doubled, total lipid content remained stable indicating that casein did alleviate the effects but did not prevent fatty infiltration (Shakahnovskava, 1953).

34. A group of studies were carried out in 1937 where mixtures of different chloronaphthalenes were given to rats in the diet. 3 g trichloronaphthalene with traces of tetra- congeners was given to 10 rats each day. No loss of weight occurred though one animal died due to a respiratory infection. Slight changes were observed in the liver. 0.5 g of a mixture of tetra- and penta- congeners was given daily to 10 animals and the amount was decreased proportionately as animals were terminated from the experiment. All animals were dead after 2 months. Gross and microscopic examination revealed that the liver was the only organ affected. Swelling, hypergranulation, hyaline inclusions and vacuolations were observed in the liver cells. Some cells were necrotic and a large accumulation of fat had occurred. Another 10 rats received 3 g penta- and hexa- congeners. All animals were dead or moribund after 1 month and had shown a loss of weight from the start of the experiment. Liver was again the only organ affected and lesions were similar to those seen in animals given tetra- and penta- congeners but more severe. Penta- and hexa- congeners were also given as a mix with chlorinated diphenyl. 3 g per day was given for 12 days before termination of dosing and all animals were dead after a further 3 weeks. Autopsy showed extremely severe lesions of the liver. A 0.5 g dose every other day caused a loss of weight and liver lesions. In addition all these compounds were also administered to rats and rabbits by stomach tube at a dose which a 50 kg man would inhale over an 8 hour working day when exposed to 20

mg/m^3. The results were similar to those given above though the lesions were less severe. Subcutanteous injection of a dose corresponding to 4 mg/m^3 again showed similar results (Drinker *et al.*, 1937).

35. Male Wistar rats were administered a mixture of tetra- (54%), penta- (8%), hexa- (23%) and heptachloronaphthalene (14%) by oral gavage at doses of 0, 1, 10 or 100 mg/kg b.w./day for 7, 14 or 21 days and blood samples and whole liver were analysed. At doses of 10 mg/kg b.w./day and above there was a substantial (50%) decrease in fodder and water consumption with related decrease in body weight by 15-30% of their initial body mass, so much so that the 100 mg/kg b.w./day group was terminated after 14 days. Relative liver weight was increased in all treated groups (118 - 151% of control values) with an increase in total CYP levels and CYP1A activity unrelated to the dose applied. No significant change in serum ALT activity was detected while a slight significant increase was seen in serum SDH activity after 14 days administration of 100 mg/kg b.w./day and 21 days administration of 10 mg/kg b.w./day. Hepatic GSH levels were reduced above 10 mg/kg b.w./day after 14 days and hepatic malondialdehyde levels were increased above 10 mg/kg b.w./day at all time points and in the 1 mg/kg b.w./day treated group after 21 days. The authors suggested that the changes in hepatic GSH and malondialdehyde indicate induction of lipid peroxidation and oxidative stress in the livers of these rats (Kilanowicz *et al.*, 2008).

36. Investigations on a mixture of penta- and hexachloronaphthalenes showed that rats receiving daily doses of 5 or 10 mg tolerated this up to 5 weeks at which time dosing stopped. Those receiving 15 mg/day all died within 3 weeks of initiation of dosing (Cleary *et al.*, 1938).

37. Groups of 104 female rats of the Carworth Farms-Elias strain were given 0, 0.002%, 0.0063% or 0.02% hexachloronaphthalene in the diet for 12 weeks (equivalent to 0, 2, 6.3 and 20 mg/kg b.w./day respectively). An additional group received 0.02% in the diet (20 mg/kg b.w./day) for 4 weeks and then were put back on the control diet for 4 weeks. On the day before the end of the 1st, 4th and 8th weeks of dosing, 26 animals from each group were used for subanalyses either looking at hexobarbital response or response to pentylenetetrazol. The day after these tests, animals were autopsied. Animals receiving 0.02% in the diet (20mg/kg b.w./day) showed decreased food consumption, depression of body weight and weight gain and a dose response was observed with 0.0063% in the diet (6.3 mg/kg b.w./day) having marginal response and little effect being observed in animals receiving 0.002% in diet (2 mg/kg b.w./day). Relative liver weight was increased in a dose dependent manner above that of controls. Relative kidney weight was less sensitive with only the high dose showing an increase compared to control (Weil and Goldberg, 1962).

38. Groups of 6 male weanling Holtzman rats were given Halowax 1051 (90% octa- and 10% hepta- congeners) in a vitamin A-free diet. Vitamin A was given orally or intravenously. At termination, liver, kidney and serum vitamin A levels were determined. Preliminary results showed that 0.002% in the diet (equivalent to 2 mg/kg b.w./day) could significantly depress vitamin A levels in the liver. In the first experiment of this series, rats were partially depleted of vitamin A and then given a daily supplement for 10 days to build up uniform supplies. After 22 days of receiving

diet with 0.05, 0.2 or 0.5% compound (50, 200 or 500 mg/kg b.w./day), vitamin A stores in liver and kidney were significantly depleted at all doses and in a dose dependent manner. Serum levels increased with increasing dose. In a second study on weanling rats receiving 0.05% or 0.30% of the compound in the diet (50 or 300 mg/kg b.w./day), a decrease in liver and kidney vitamin A was observed but no significant effect on liver vitamin E was detected. Young rats receiving 0.3% in the diet (300 mg/kg b.w./day) showed no changes in serum vitamin A or E levels. At autopsy it was noted that animals dosed with the compound receiving a vitamin A free diet had livers that appeared normal or only slightly yellow whereas those receiving a normal diet had mottled, light coloured livers (Deadrick *et al.*, 1955).

39. One study compared the effects of a mixture of tetra-, penta- and hexachloronaphthalene congeners with 62% chlorine content in rats and hamsters. Groups of Sprague Dawley rats (n=3-4) were given diets containing 25, 50, 100 or 400 mg/kg of this mixture for 8 weeks before autopsy (equivalent to 2.5, 5.0, 10.0, 40.0 mg/kg b.w./day). Liver vitamin A levels were determined at sacrifice. 2 animals in the high dose group died before the end of the experiment. Average weight gain and liver weight was substantially decreased in the high dose group compared to the other groups and liver vitamin A content decreased above 50 mg/kg feed. A group of 9 hamsters received diet containing 0.4% of the PCN mixture (400 mg/kg b.w./day) and another group of 8 hamsters received the same amount in a diet with decreased vitamin A content. Only 1 animal from these two groups survived to the end of the experiment and all animals lost weight. Liver weight and liver vitamin A content at termination was substantially lower than those on basal feed (Schoettle *et al.*, 1955).

40. Six guinea pigs were given doses of synthetic pentachloronaphthalene from 2.5 to 20 mg/kg b.w./day for 6 days per week until death. Administration lasted between 4 and 48 days with animals losing weight until death. Autopsy showed fatty degeneration of the liver in most of the animals. Oral administration of 10 to 20 mg/kg b.w./day Haftax 1 (technical PCN with 52% chlorine content) to 5 guinea pigs caused striking weight loss associated with lack of appetite and nasal and oral discharge and conjunctivitis. Death occurred between day 9 and 19. Autopsy showed dilation of the right heart, congestion and oedema of the lungs, fatty degeneration in the liver, and catarrhal gastroenteritis. This experiment was repeated with Haftax 2 (another technical PCN with 65% chlorine content). Results were as for Haftax 1 though death occurred earlier, between day 5 and 9 (Bentz and Herdmann, 1955).

41. Nine pigs were given a balanced ration with 400 units vitamin A per pound. Of these animals, 6 received 22 mg/kg b.w. hexachloronaphthalene in gelatin capsules per day for 8 or 9 consecutive days. Plasma vitamin A levels were assessed prior to the start of the experiment, 14 days after initiation of treatment and at necropsy. 14 days after initiation, treated pigs had an average plasma vitamin A value of 6.6 µg / 100 ml compared to controls with 27.7 µg / 100 ml ($p < 0.001$). At necropsy there was no significant difference in plasma vitamin A levels. Depression, anorexia and ataxia were observed in treated pigs. There was severe depression of weight gain and 2 animals lost weight, however no other signs characteristic of bovine hyperkeratosis were observed. The liver had slight pale brown discoloration and showed a limited amount of swelling and a slightly thickened intestinal wall. Histopathologic examination showed degenerative liver changes with swollen granular parenchymal cells with cloudy swelling and distended sinusoids in some sections. Dilatation of the

tubules, granulation and swelling of the parenchymal cells with a loss of cytoplasm and amorphous proteinaceous cast formation in the tubules were observed in the kidneys. There were some hyperplastic changes in the luminal surface of the vagina. Inflammation was observed in the duodenum with changes suggesting subacute interstitial duodenitis (Huber and Link, 1962).

42. A study was carried out in swine to examine the effect of oral exposure via gelatin capsules containing hexachloronaphthalene. 5 swine (70 to 80 kg b.w.) were given a total dose of 110 mg/kg b.w., 5 pigs (15 to 25 kg b.w.) were given a total dose of 132 to 220 mg/kg b.w. and 7 pigs (15 to 29 kg b.w.) were given a total dose of 176 to 198 mg/kg b.w over up to 10 days. Necrosy was carried out when animals were moribund or between day 36 and 52. Blood vitamin A levels were assessed prior to initiation of PCN administration and 14 days after the initial dose. The swine given 110 mg/kg b.w. showed no reaction in vitamin A, body weight or following gross and microscopic examination of the liver and kidney after 52 days. In pigs receiving doses from 132 to 220 mg/kg b.w., there was a reduction in vitamin A levels at all doses with those receiving above 154 mg/kg b.w. showing signs of deficiency. A total dose of 176 mg/kg b.w. caused growth retardation and those receiving above this dose died or became moribund. Of the 7 pigs receiving 176 to 198 mg/kg b.w., one did not appear fatally poisoned and showed a slight depression of plasma vitamin A. The other pigs died or were killed when moribund. The typical signs of hyperkeratosis as seen in cattle were not observed. Gross lesions in moribund or dead animals were swelling and haemorrhage of the liver some with yellow discolouration, thickening of the intestinal wall, mild gastritis, eyelid oedema and swelling of the epididymes. While some animals showed deficiency in vitamin A after 14 days, this returned to the normal range later (Link *et al.*, 1958).

43. Groups of 5 ewes were given 0, 1.1 mg/kg b.w./day, 11.0 mg/kg b.w./day or 27.5 mg/kg b.w./day Halowax 1014 by gelatin capsule until death. The group receiving 1.1 mg/kg b.w./day required an average total dose of 117 mg/kg to produce death which occurred between day 90 and 135 when the final animal was sacrificed. The group given 11.0 mg/kg b.w./day were treated for an average of 27 days (range 23-35) and received an average total dose of 301 mg/kg before death occurred. The high dose group survived for 20 days (range 16 to 25 from 4 animals) and received an average total dose of 563 mg/kg. After 5 days dosing, animals in the mid and high dose groups showed decreased plasma vitamin A levels, however the levels determined were not indicative of severe avitaminosis A. In all groups, excessive nasal discharge, weakness, loss of weight and appetite were observed with signs being of longer duration before death in the lower dose group. All treated animals had liver damage, varying degrees of gastrointestinal haemorrhage and increased pleural and/or peritoneal fluid. Severity of the lesions increased with dose. High dose group showed congestion and ecchymoses throughout the GI tract and the livers were swollen, congested and friable with a mottled appearance as areas of haemorrhage were intermingled with cream coloured areas suggesting fatty degeneration. Bile ducts showed thickening and the gall bladders had ecchymoses in the fundi. Kidneys were congested with reddish brown streaks in the medulla. The larynx, trachea and bronchi were congested and had submucosal haemorrhage and frothy mucus was found in the lumen. Increased fluid was observed in the pericardial sacs, the spleens were congested and blood did not clot. 1 animal had increased quantity of peritoneal fluid and 1 animal had uterine haemorrhage and another

placental separation. Another 2 animals showed degeneration and haemorrhage of the adrenal glands. In the mid dose group, the livers of 2 ewes had an appearance of early cirrhosis, otherwise lesions were similar to those of the high intake group though in some instances less severe. The low dose group showed less severe lesions again though cirrhosis of the liver was more obvious with a nodular appearance on the surface. In addition, large quantities of straw coloured fluid were present in the peritoneum and mesenteries and omenta were thickened and contained petechiae. No lesions of the kidneys, hearts or spleens were observed in this group. Histopathological findings in the mid and high dose group were similar. There was marked congestion, haemorrhage and necrosis in the livers, congestion of the gallbladders and coagulation necrosis of the mucus membranes in the bile ducts. Congestion of the kidneys occurred particularly in the medulla, with heavy deposits of yellowy brown granules along the blood vessels. Endometrial congestion and fragmentation of the uterine epithelium was observed. Bronchial exudate and congestion were observed in the lungs with some areas of haemorrhage and atelectasis. Cloudy swelling, myocardial hyperemia and small haemorrhages in the coronary vessels in the epicardia were observed in the hearts. Marked passive hyperemia and atrophy of the malpighian corpuscles were identified in the spleens. Adrenal glands showed hyperemia and degeneration of the cortex. Congestion, small haemorrhages and degeneration of the mucosa was found in the abomasum and colon. Animals receiving the low dose showed similar lesions but of more chronic form. The authors noted that the hyperkeratosis and severe depression of plasma vitamin A levels observed following exposure of cattle were not observed in the sheep in this experiment (Brock *et al.*, 1957).

44. A group of calves were given di- to octachlorinated naphthalenes (n=2-5 per compound) in gelatin capsules for 7 to 13 days with some receiving further treatment on days 23 to 30. The total dose administered was between 0.5 and 4.6 grams. The animals were kept till they became weak or moribund. Calves receiving di- and trichloronaphthalenes did not develop signs of hyperkeratosis. Two of the 3 calves receiving tetrachloronaphthalenes developed mild reversible signs which reoccurred after repeat administration. After 150 days observation, no ill effects were found. The third animal receiving tetrachloronaphthalenes showed no gross or microscopic lesions after 81 days. The calf receiving the lowest total dose (11.9 mg/kg) of pentachloronaphthalenes showed signs of hyperkeratosis from days 7 to 60 but these were transient and by day 150 no visible signs or external lesions could be observed. The other two calves receiving pentachloronaphthalenes showed signs of hyperkeratosis and were autopsied at day 22. All 5 calves receiving hexa- and the two calves receiving heptachloronaphthalenes showed signs of hyperkeratosis and had to be autopsied between days 15-29 for the hexa- and days 34-39 for the heptachloronaphthalenes. In addition, these calves also had enlarged, grey kidneys with striations in the cortex, liver effects (light colouring on the surface and parenchyma in hexa- treated and fibrosis in hepta- treated animals), distention of the gall bladder, ulcers or erosion of the mucosa in the abomasum near the pylorus and wart-like proliferations in the mouth. Hexa- treated animals also showed inflammation of the small intestine and hepta- treated animals had nodules in the vaginas. Two octachloronaphthalene treated animals showed no signs while one other animal showed effects on milk for 50 days but with no visible effects for the remainder of the 125 day period. One animal was moribund by day 21. At autopsy, the hooves were indented, wart-like proliferations were present on the tongue, kidneys were enlarged

and pale and light-coloured areas were present on the liver and the gall bladder was distended with thickened walls. Microscopic examination of the lesions found showed dilatation of the kidney tubules, bile duct proliferation with cellular infiltration and degeneration in most liver sections. Dilatation of the glands of the gall bladder and squamous metaplasia of the epididymes were occasionally present (Bell, 1953).

45. Pentachloronaphthalene was administered orally via gelatin capsules to 3 female cows. One received 15 g/day for 13 days and died on day 17 while the other two received 2 g/day for 10 days, followed by 4 g/day for a further 10 days, then 6 g/day for 10 days and finally 8 g/day for the final 10 days. Over the total period, these animals each received 200 g pentachloronaphthalene. Excessive lacrimation, diarrhoea, polyuria, salivation and serous discharge from the nostrils followed by chronic cough, poor appetite and development of red macules in the buccal cavity were observed. By day 35, hyperkeratosis had developed on the sides of the neck, across the withers and around the mammary glands. Proliferative areas on the gums, lips and hard palate, ulcers of the abomasum, swollen and hard pancreas were observed at autopsy. The liver edges were rounded and thickened, the gall bladder was distended with thickened walls and contained dark, sticky, viscid bile and mucous cysts were found in the large bile ducts. The kidneys were enlarged and contained subcapsular cystlike structures in the cortex. Keratinization of the hair follicles and accumulation of keratinized material was observed microscopically. Central lobular degeneration of the liver and bile duct proliferation and dilation of the walls of the gall bladder was also seen. Cystic dilation of the collecting tubules and fibrosis were observed in the kidneys (Sikes and Bridges, 1952).

46. Experiments carried out on cows and calves administered hexa- or octachloronaphthalene orally in a gelatin capsule. In the first experiment, one calf was given 3 grams hexachloronaphthalene daily and was autopsied at day 27 due to being in a morbid state. The other calf was given 1 g per day for 30 days followed by 2 g per day for a further 30 days, receiving a total of 90 g over 60 days. In the second experiment, a cow with a 2 month old bull calf was given 5 g octachloronaphthalene per day for 18 days. The calf was allowed to nurse but was otherwise kept separate and no compound was given directly to the calf. In the other part of this second experiment, an 8 month old bull was given 1 g octachloronaphthalene for 13 days. Signs observed were excessive lacrimation, diarrhoea, polyuria, salivation and discharge from the nostrils. By the day 40, the animals had a chronic cough, poor appetite and macules in the buccal cavity, lips and nose. Necropsy showed generalized hyperkeratosis, inflamed, swollen and oedematous abomasum, enteritis though the upper third of the small intestine, hard, swollen and firm pancreas. The edges of the liver were rounded and thickened, the gallbladder was distended with thickened walls and filled with thick, viscid, dark coloured bile, mucous cysts were observed in the large bile ducts. In the kidney cortex, clear subcapsular cyst like structures were observed. In the experiment with the cow and calf, the cow showed reduced milk flow by day 7 and cessation of flow by day 29. Autopsy on day 42 showed proliferations of the bile ducts, mouth and nose, dehydration of the skin but no hyperkeratosis. Autospy of the calf, which had received milk from the dairy herd when there was not enough from the dam, on day 50 showed moderate hyperkeratosis (Sikes *et al.*, 1952).

47. Seven calves given a 3% solution in mineral oil of a highly chlorinated naphthalene for between 7 and 29 days, with a total dose of 15 to 170 g, showed lesions typical of bovine hyperkeratosis. Some animals had rapid onset of disease while in others chronic disease did not develop and a reversal of induced effects was observed. Autopsy showed gross changes in the digestive tract, liver, gall bladder and urinary tract and other gross and microscopic effects as described in the Bell 1953 study (Bell, 1952).

48. Groups of 16 Broad Breasted Bronze turkey poults (8 male and 8 female) received diet containing 0, 5, 10, 20, 50 or 100 ppm Halowax 1014 (penta- and hexa-congeners) for 40 days from one day of age. Animals in the 5 ppm group showed an average decrease of 33% in body weight gain compared to controls. This effect increased with increasing dose and all males in the 50 ppm group and all animals in the 100 ppm group died before the end of the experiment. The results showed that males were more susceptible to the effects than females. Gross autopsy showed dark enlarged livers (Pudelkiewicz, 1958).

49. Groups of 10 (5 male and 5 female) Broad Breasted Bronze turkey poults received 125 ppm Halowax 1051 (mainly octa- congener) in the diet from 1 day of age. After 35 days weight gain was higher in the treated group (526 g) than the controls (486 g) but no other differences were observed between the two groups (Pudelkiewicz, 1958).

50. A group of experiments were carried out on New Hampshire Chickens. Day old chicks were raised in brooders. The first experiment was looking at the plasma vitamin A response. Groups of 10 male and 10 female day old chicks were put on a low vitamin A diet till day 12 when they were transferred to a diet with 4400 I.U. vitamin A per kg with 0, 4, 20, 100, 500 or 2500 ppm Halowax 1014 for 40 days. Another group was transferred to a diet containing 17600 I.U vitamin A per kg and 500 ppm Halowax 1014. At the end of the experiment chicks were sacrificed and blood and liver vitamin A levels were analysed. Diets containing 4 and 20 ppm Halowax 1014 were tolerated, at 100 ppm weight gain was restricted and the diet containing 2500 ppm caused fatality of all chicks within 2 weeks. The liver was enlarged and fibrotic and straw coloured fluid was present in the cavities, starting in the pericardial sac. Animals were also suffering from anaemia. Vitamin A concentration in the blood plasma was variable though a decrease was noted at 500 ppm. Liver storage of vitamin A was depressed with increasing dose. An increase in blood vitamin A content and liver storage of vitamin A was seen when the diet was supplemented with vitamin A. Feather pigmentation varied with increasing dose. In the second experiment, day old chicks were placed on a diet containing 100 ppm Halowax 1014. Chicks were sacrificed after 35 days and blood samples were analysed for vitamin A, sodium, potassium, uric acid, creatinine and cholesterol. Results showed significant increase ($p=0.05$) in serum uric acid concentration and very significant ($p=0.01$) decrease in haematocrit. No significant changes in other parameters were observed. The third experiment looked at the effect of Halowax 1014 on egg production and hatchability. Diets containing 0, 4, 20 or 100 ppm Halowax 1014 were fed to day old chicks for 28 weeks. At sexual maturity, pullets and cockerels were separated into individual cages and egg production was recorded after acclimatisation for 1 week. No differences were observed at a dose of 4ppm in the diet. At 20 ppm, no differences in the rate of egg production were observed but

the hatchability of the eggs was markedly decreased. No eggs were produced by animals receiving 100 ppm in the diet, animals showed pale and underdeveloped combs and wattles, and enlarged livers and straw coloured fluid in the pericardial sac and peritoneal cavity were found on autopsy. The progeny of the chickens receiving up to 20 ppm Halowax 1014 showed a reduction in weight gain after 28 days compared to controls (Pudelkiewicz, 1959).

51. 40 mice were exposed to 0.05 - 2 mg trichloronaphthalene vapour/l air for 2 hours per day. During the first days of exposure slight excitation in behaviour was observed. Animals died between days 7 and 80 with loss of weight and dermal effects, namely blackening and necrosis of the tail and ears, swelling and reddening of the paws with necrosis of the digits. Pronounced liver effects, fatty degeneration, hyperplasia of the reticuloendothelium, and hyperemia, were observed along with less severe effects in the kidney, parenchymatous degeneration of the tubular epithelium, and hyperemia. Vascular effects were observed in the lungs along with inflammation of the trachea, bronchi and bronchioles. Intercurrent myocarditis and hyperplasia of the splenic reticuloendothelium were also observed. As part of the same set of experiments, groups of 5 rats exposed for 60 or 300 hours to 0.05 - 2 mg trichloronaphthalene vapour/l air for 2 hours per day. All rats survived this exposure and a slight increase in excitability was the only change in behaviour. After 1.5 to 2 months, swelling and reddening followed by blackening of the facial region and nose occurred. This progressed to ulceration. After cessation of administration, progression of skin effects stopped. Overall, the pathological effects in the rats were as those seen in the mice but less severe (Shakahnovskava, 1953).

52. Inhalation experiments on rats with whole body exposure were carried out with trichloronaphthalenes containing a trace of tetrachloronaphthalene, a mixture of penta- and hexachloronaphthalene and a mixture of 90% penta-and hexachloronaphthalene with 10% chlorinated diphenyl. Exposure was between 1.16 and 1.37 mg/m^3 for 16 hours per day, 6 days per week. Animals receiving trichloronaphthalene appeared normal throughout the experiment. At autopsy, slight swelling of the liver was present and microscopic examination showed swollen and hypergranular liver cells. Those receiving the mixture of penta- and hexachloronaphthalene appeared normal. Autopsies were performed on animals killed every 6 weeks. Liver changes, swollen cells, slight granulation and hyalinization, were observed after the first 6 weeks and became more advanced with time. After 4 months no further advance in disease state occurred. Similar signs were seen when 10% chlorinated diphenyl was added to this mixture. There was some evidence for recovery 2 months after exposure ceased but this was not complete. Higher dose experiments (10.97 mg/m^3) showed that trichloronaphthalenes also had potential to induce liver effects but at a much higher concentration than the more highly chlorinated congeners. Similarly high dose experiments (8.88 mg/m^3) with the penta- and hexachloronaphthalene mixture showed much more severe liver effects with loss of weight and deaths from day 8, many had jaundice. Microscopic examination showed central fatty degeneration and cell necrosis. Pronounced changes were still present 35 day after cessation of exposure. An experiment was also carried out on the penta- and hexachloronaphthalene mixture with or without PCBs decreasing the daily exposure to 8 hours per day and using between 1.44 and 1.66 mg/m^3. These animals did not show signs of illness though there were some microscopic liver changes from 6 weeks (Drinker *et al.*, 1937).

53. Groups of 5 rabbits (approximately 2 kg each) were given 30 mg of mixtures of tri- and tetra-, tetra- and penta- or penta- and hexachloronaphthalene by subcutaneous injection daily until death. In addition 2 groups were given sublimates of the tetra-/penta- mixture given off at 192 ^{o}C and the penta-/hexa- mixture given off at 172 ^{o}C. No animals died in the groups given the tri-/tetra- mixture or the sublimates of the tetra-/penta- and no pathological conditions were found on autopsy. Animals receiving the tetra-/penta- mixture died between day 12 and 15 and those receiving the penta-/hexa- mixture died between day 12 and 26. Those receiving the sublimate of the penta-/hexa- mixture died between day 9 and 14. In these animals the liver was large, dark red and with many opaque yellow areas. There was loss of lobulation and in areas the parenchyma had disappeared and the stroma collapsed. The gall bladder was distended and there was fluid in the peritoneal cavity. Histologic examination of the liver showed widespread necrosis, calcification of the stroma and multinucleated giant cells. There was an indication of bile canaliculi distention (Flinn and Jarwick, 1936).

Reproductive and Developmental studies

54. One study has been carried out in which 7 pregnant Wistar rats were given 1,2,3,4,6,7-hexachlorinated naphthalene at 1 µg/kg b.w./day by gavage on gestational days 14, 15 and 16. On the day of birth, offspring were counted, sexed and examined for gross malformations and litters were reduced to five males and five females. On postnatal days (PND) 31, 48, 62 and 89, one male from each litter was euthanized and the testes, epididymis, ventral prostate and seminal vesicles were removed and weighed. Homogenization-resistant spermatids (advanced spermatids) from the testes and the sperm collected from the cauda epididymis were counted and sperm motility was determined. There were no significant differences between the treated and control groups with respect to offspring body weight, anogenital distance, survival during lactation and day of eye opening. Testis and epididymis weights were not significantly different between the groups. Caudal sperm count on PND 62 was increased in the treated group to 180% ($p < 0.01$) of the control value though there was no significant difference on PND 89. There was no difference in sperm motility between the groups. On PND 48, the homogenization-resistant spermatid count in the treated group was 160% ($p < 0.05$) of the control value. On PNDs 62 and 89, there were no significant differences between control and treated groups. Histological examination showed no significant differences in the percentage of postmeiotic seminiferous tubules on PND 48, though the treated group had a higher percentage of tubules with step 19 spermatids. On PND 31, the treated group had a higher percentage of postmeiotic tubules, 190% ($p < 0.05$) of the control value with a much higher percentage of tubules containing step 7 or more advanced spermatids. Serum concentrations of LH reached a plateau and FSH reached their maximum levels on PND 48 for control group and on PND 31 in the treated group. The authors hypothesised that administration of 1,2,3,4,6,7-hexachlorinated naphthalene accelerated onset of sperm production in the testis. The possibility of effects of litter size on spermatogenesis was investigated, but no effect was found. The authors acknowledged there could be an effect of intrauterine position, which was not determined in this study, however using male:female ratio at birth as a representative measure, this was not found to have an effect on the early onset of spermatogenesis.

The early secretion of FSH and LH from the pituitary was considered to be responsible for the early onset of spermatogenesis (Omura *et al.*, 2000).

55. A study was carried out to assess the effects of a mixture of penta- and hexachloronaphthalene on spermatogenesis in a yearling bull. A daily dose of 50-200 mg was given over a 7 week period. A total dose of 1.8 g was given. The bull was then kept for a year after cessation of dosing. Plasma vitamin A levels were assessed to regulate the dosage given. Semen was collected and assessed on a weekly basis for the first 4 months and then at monthly intervals. 10 days after the final dose was administered, the left testis was removed to determine testicular degeneration. Prior to termination the bull was bred naturally with one heifer and artificially with another. Plasma vitamin A levels decreased upon administration of the mixture and continued to decrease for 2 weeks after cessation of dosing. After this time, levels increased steadily. Acute signs of hyperkeratosis were observed and the skin of the neck thickened after 1 month. The disease progressed for approximately 5 months, however noticeable improvements in condition were observed after 8 months. Libido decreased slightly over the course of the disease. No spermatozoa were present in the semen after 2 months and this continued for 6 months. Spermatic cytology showed increased in detached heads, number of spermatozoa with protoplasmic droplets and percentage with abnormally shaped heads as levels of spermatozoa declined. As recovery progressed, there was a decrease in these abnormalities. Microscopical analysis of the left testis showed degeneration of the seminiferous epithelium and squamous metaplasia of the head of the epididymis. Both heifers mated with the bull became pregnant, first time after natural breeding in one cow and after 2 inseminations in the other. At slaughter the bull showed thick skin over the upper two thirds of the body, deeply indented horns, a deep scar at the base of the tongue, fibrosis of the pancreas and small cysts in the gall bladder (Vlahos *et al.*, 1955).

Epidemiology studies

56. In a 1937 report, PCNs and PCBs were associated with three fatal cases of jaundice at the Halowax corporation. In all three cases, there were no reports of precipitating causes. Two patients had yellow atrophy of the liver (no report is given for the third man) of whom one had chloracne along with a number of his co-workers, none of whom had developed any other health effects (Drinker *et al.*, 1937).

57. A further three case reports of fatal yellow atrophy of the liver were reported in 1939. A 17 year old female had been working on soldering condensers. As a result she was exposed to fumes from tri- and tetrachloronaphthalenes and she worked in proximity to fumes from higher chlorinated congeners. She developed signs and symptoms of jaundice and acneform eruptions two months after starting work and after working for 7 months she was admitted to hospital with weakness, nausea, persistent headache and swelling of the face, hands, feet and abdomen. A 24 year old male was exposed to wax with highly chlorinated naphthalenes from wire coating for 6 months prior to admission to hospital with jaundice. 3 months later after an improvement in the jaundice he returned to work at which time the jaundice became intense and he showed general malaise, dizziness and loss of weight. A 22 year old male working in the same wire coating factory. He had jaundice for 2 months prior to

admission and 2 weeks before admission this had become worse and he had developed abdominal pain, malaise, nausea and vomiting (Greenburg L, 1939).

58. 12 workers in a chromium plating factory with exposure to dust of fumes of chlorinated naphthalenes were shown to have typical chloracne lesions. Another young woman suffered acute yellow atrophy of the liver leading to death following 6 months exposure. The author suggested this may have been a consequence of a previous history of endocarditis which made the liver more susceptible to the effects of chloronaphthalene (Collier, 1943).

59. Case reports of seven people coming into contact with pentachloronaphthalenes suggest that papular rast or depigmentation are the most common early symptom of susceptibility. Liver damage is a possible consequence of continued exposure however removal from contact at time of gastrointestinal symptoms led to rapid recovery (Cotter, 1944).

60. A study of 16 workers exposed to wax fumes, of which 90% was PCNs, has been reported. Exposures lasted from 1958 to 1989 though the composition of the waxes varied through this period. 6 workers were examined clinically and a further 4 workers were assessed for liver enzyme parameters (one of whom had diabetes mellitus with blindness and apoplectic insult and another had had renal and bladder cancer) and 2 others had their GGT levels measured (one of whom had laryngeal cancer). 3 workers could not be contacted and the last worker had died of stomach or colon cancer. The results of the study showed that GGT values were increased in 6 of the 16 workers, one person had increased bilirubin and another increased GPT. 2 workers had fatty livers. Other factors were not changed and chloracne was not reported. The authors concluded that for at least some of the exposed workers, the changed in liver function could be a result of the exposure to PCNs in the workplace (Popp *et al.*, 1994).

Estimates of dietary exposure

61. Dietary intake has been estimated for the population of Catalonia, Spain. Food samples were obtained in summer 2000 from markets, supermarkets and grocery stores in seven cities and analysed for tetra-, penta-, hexa-, hepta- and octa-congeners. Using previously determined daily consumption and assuming non-detects were half the limit of detection (medium bound), intake was estimated for various population groups. On a bodyweight basis, children consumed highest levels with an estimated intake of 1.65 ng/kg b.w./day and it was estimated that a 70 kg male would have a total intake of 45.78 ng/day or 0.65 ng/kg b.w./day (Domingo *et al.*, 2003). A subsequent study on the diet of elite sportsmen and women estimated that average dietary intake is 56.63 ng/day and 38.00 ng/day for elite sportsmen and women in Catalonia, Spain, respectively. For high consumers, dietary intake was 72.31 ng/day for elite sportsmen and 47.08 ng/day for elite sportswomen (Falco *et al.*, 2005).

62. In a 2003 review on PCNs as food chain contaminants, it was highlighted that frequently mono-, di-, tri- and octachloronaphthalenes had not been analysed in environmental matrices and food due to a lack of analytical capability in the

laboratories. However improved chromatographic techniques seem likely to enable separation of individual congeners and their quantification in foodstuffs. In addition, to concentrations in foodstuffs, there are also a number of studies which have analysed human body concentrations (Falandysz, 2003).

63. Dietary intake of PCNs (tri- to octachloronaphthalene congeners) from fish in two Chinese coastal cities was estimated to be 16.6 pg/kg b.w./day and 19.6 pg/kg b.w./day assuming non-detect levels were at the level of quantitation. This was determined to be equivalent to 0.00 and 0.01 pg WHO-TEQ/kg b.w./day, where the dioxin-like toxicity was estimated from the relative potency values determines in the Blankenship *et al.*, 2000 and Villeneuve *et al.*, 2000 studies (Jiang *et al.*, 2007).

64. The contribution of fish to the dietary intake of PCNs in Catalonia, Spain, in 2007 has been estimated. 42 composite samples of fish were analysed for tetra- to octachloronaphthalenes and a total PCN intake from fish for a standard male adult of 70kg was estimated to be 1.53 ng/day. Analysis on a body weight basis was also carried out with the highest consumers being boys with an intake of 0.04 ng/kg b.w./day (Llobet *et al.*, 2007). Using these data and taking into account data on tetra- to octa- congener concentrations in other food groups such as meat and meat products, fruit and vegetables, eggs, milk and dairy products, pulses, oils and fats and bakery products sampled in spring 2006, total dietary intake of PCNs for a 70 kg adult male was estimated at 7.25 ng/day or 0.10 ng/kg b.w./day. The authors noted that this was a substantial reduction from the corresponding level in 2000 of 45.78 ng/day or 0.65 ng/kg b.w./day. This was likely due to an overall decrease in consumption of the different foodstuffs as well as decreases in concentration in oils and fats, and cereals. Highest intake on a bodyweight basis in this analysis was found to be boys aged 4 to 9 years old (Marti-Cid *et al.*, 2008). Children's intake from fish and seafood from the same area, surveyed in 2005, was estimated to be 0.97 ng/day for boys and 0.52 ng/day for girls (Marti-Cid *et al.*, 2007).

65. Samples of adipose tissue and liver from Swedish subjects (5 male and 2 females, aged 47 – 80 years old) who had suffered sudden death were analysed for PCN content. No previous history of exposure was available. Results showed that liver generally contained higher levels than adipose tissue and a range of tetra- to hexa- congeners were found (Weistrand and Noren, 1998).

66. An analysis of human milk samples from Sweden collected between 1972 and 1992 has showed a decrease to 16% of the 1972 level by 1992 with a half life of 8 years (Noren and Meironyte, 2000).

67. Exposure to the UK population has not been determined as part of the investigation presented in this paper, as the small number of samples taken means that the concentrations are unlikely to be representative.

Toxic Equivalency Factors

68. Due to their structural similarity to dioxins, many papers have suggested deriving Toxic Equivalency Factors (TEFs) for the different PCN congeners to enable determination of 2,3,7,8-TCDD Equivalents (TEQ).

69. According to van den Berg *et al.* (2006), the criteria for inclusion in the TEF concept are that a compound must:

- Show a structural relationship to the PCDDs and PCDFs
- Bind to the Ah receptor
- Elicit AhR mediated biochemical and toxic responses
- Be persistent and accumulate in the foodchain

70. During the most recent WHO re-evaluation of the TEFs for dioxins and dioxin-like compounds, it was suggested that PCNs and their brominated counterparts might be considered for inclusion in the concept. This was on the basis that *in vivo* studies have shown induction of dioxin-like effects and AhR activities analogous to PCDDs and PCDFs have been observed in *in vitro* studies though the PCNs are less potent. It was noted that the potential for contamination of PCNs with small amounts of other dioxin-like compounds has potential to influence the effects seen and that the dioxin-like toxicity should be evaluated using very pure samples. In addition, human exposure data were felt to be lacking to determine whether the PCNs are relevant in terms of the TEQ dietary intake (van den *et al.*, 2006). These features and the planar structure and lipophilicity of PCNs and other similarities with dioxins and dioxin-like compounds have also been commented on by other authors e.g. (Falandysz, 1998) and (McKinney and McConnell, 1982).

71. Several papers discuss the use of *in vitro* assays such as the ethoxyresorufin O-deethylase (EROD) activity in fish and rat cell lines (Behnisch *et al.*, 2003; Hanberg *et al.*, 1990; Hanberg *et al.*, 1991; Villeneuve *et al.*, 2000; Villeneuve *et al.*, 2001), DR-CALUX (Behnisch *et al.*, 2003), H4IIE-luciferase (Blankenship *et al.*, 2000; Villeneuve *et al.*, 2000) and aryl hydrocarbon hydroxylase (AHH) activity (Hanberg *et al.*, 1990) assays to compare the activity of individual PCNs or mixtures of PCNs with 2,3,7,8-TCDD and to derive relative potencies (REPs). Aryl hydrocarbon receptor (AhR) binding assays have also been carried out (Hanberg *et al.*, 1990).

72. *In vivo* assays have also been used to compare the activity of PCNs or mixtures by analysing liver enzyme activities, such as AHH (Campbell *et al.*, 1983; Cockerline *et al.*, 1980) EROD (Campbell *et al.*, 1983; Mãntylã and Ahotupa, 1993) or pentoxyresorufin O-deethylase (PROD) (Mãntylã and Ahotupa, 1993). In addition experiments have been carried out evaluating liver vitamin A, α-tocopherol and GSH content and liver GSH-S-transferase, GSH peroxidise, catalase, superoxide dismutase and hexose monophosphate shunt activity (Mãntylã and Ahotupa, 1993).

73. A number of authors have determined REPs compared to 2,3,7,8-TCDD experimentally in *in vitro* studies and these are summarised in Table 1. The available data have also been used to attempt *in silico* derivation of REP values for all PCNs, for example, a recent paper has used *in vitro* H4IIE EROD and luciferase assay data along with a large number of chemical descriptors to determine REP values for all the chloronaphthalene congeners. The researchers then derived TEF values relative to 2,3,7,8-TCDD (Puzyn *et al.*, 2007). A similar paper (Falandysz and Puzyn, 2004), presented a quantitative structure activity relationship (QSAR) technique used to predict EROD and luciferase inducing potency of the 75 congeners based on chemical descriptors and the values quoted in the Falandysz (2003) review. The authors also suggested reasons for the variation in the activity of higher chlorinated

naphthalenes based on their chemical properties such as symmetry, the highest occupied molecular orbital and the number of vicinal carbon atoms unsubstituted with chlorine. In another paper by Olivero-Verbel *et al.* (2004), the authors used an *in silico* approach with available H4IIE-luciferase experimental data to create a prediction model for activity of the other congeners. The derived model suggested that the atomic charges of the two carbons fusing the rings and the number of chlorine atoms on the molecules are important for determining the activation of AhR (Olivero-Verbel *et al.*, 2004).

Table 1: Relative Potency of PCN congeners compared to 2,3,7,8-TCDD determined in *in vitro* studies.

Congener	**Assay**	**Relative Potency**	**Ref**
1-monoCN	DR-CALUX in recombinant H4IIE cells	EC5: $5.0x10^{-5}$ EC20: $3.0x10^{-5}$ EC50: $1.7x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<6.4x10^{-6}$	*a*
2-monoCN	EROD: PLHC-1 fish hepatoma cells	$<7.1x10^{-7}$	*b*
	EROD: H4IIE recombinant cells	$<1.1x10^{-7}$	*b*
	DR-CALUX in recombinant H4IIE cells	EC5: $2.7x10^{-5}$ EC20: $2.6x10^{-5}$ EC50: $1.8x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.5x10^{-6}$	*a*
1,2-diCN	DR-CALUX in recombinant H4IIE cells	EC5: $<2.9x10^{-7}$	*a*
1,4-diCN	EROD: PLHC-1 fish hepatoma cells	$<1.6x10^{-7}$	*b*
	EROD: H4IIE recombinant cells	$2.6x10^{-8}$ to $3.6x10^{-10}$	*b*
	Luciferase assay: H4IIE recombinant cells	$1.2x10^{-7}$ to $1.0x10^{-8}$	*b*
	DR-CALUX in recombinant H4IIE cells	EC5: $3.0x10^{-5}$ EC20: $5.0x10^{-5}$ EC50: $3.5x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.6x10^{-6}$	*a*
1,5-diCN	DR-CALUX in recombinant H4IIE cells	EC5: $<1.2x10^{-6}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<6.6x10^{-7}$	*a*
1,8-diCN	DR-CALUX in recombinant H4IIE cells	EC5: $1.5x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.7x10^{-6}$	*a*
2,3-diCN	DR-CALUX in recombinant H4IIE cells	EC5: $3.7x10^{-5}$ EC20: $4.1x10^{-5}$ EC50: $2.7x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<5.9x10^{-6}$	*a*

Congener	Assay	Relative Potency	Ref
2,7-diCN	EROD: PLHC-1 fish hepatoma cells	$<1.6 \times 10^{-6}$	*b*
	EROD: H4IIE recombinant cells	$<2.6 \times 10^{-7}$	*b*
	Luciferase assay: H4IIE recombinant cells	$<4.2 \times 10^{-7}$	*b*
2,4-OH-CN	EROD: PLHC-1 fish hepatoma cells	$<7.4 \times 10^{-7}$	*b*
	EROD: H4IIE recombinant cells	5.5×10^{-8} to 7.6×10^{-9}	*b*
	Luciferase assay: H4IIE recombinant cells	$<1.9 \times 10^{-7}$	*b*
1,2,3-triCN	DR-CALUX in recombinant H4IIE cells	EC5: $<4.4 \times 10^{-6}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<2.0 \times 10^{-6}$	*a*
1,2,7-triCN	EROD: PLHC-1 fish hepatoma cells	$<3.8 \times 10^{-6}$	*b*
	EROD: H4IIE recombinant cells	$<6.1 \times 10^{-7}$	*b*
1,2,3,4-tetraCN	DR-CALUX in recombinant H4IIE cells	EC5: $<2.3 \times 10^{-6}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.6 \times 10^{-6}$	*a*
1,2,5,6-tetraCN	DR-CALUX in recombinant H4IIE cells	EC5: $<4.1 \times 10^{-7}$	*a*
1,2,4,7-tetraCN	EROD: PLHC-1 fish hepatoma cells	$<2.2 \times 10^{-6}$	*b*
	EROD: H4IIE recombinant cells	$<3.5 \times 10^{-7}$	*b*
	Luciferase assay: H4IIE recombinant cells	$<5.7 \times 10^{-7}$	*b*
1,3,5,7-tetraCN	EROD: PLHC-1 fish hepatoma cells	$<2.2 \times 10^{-5}$	*b*
	EROD: H4IIE recombinant cells	$<3.5 \times 10^{-6}$	*b*
	Luciferase assay: H4IIE recombinant cells	$<5.7 \times 10^{-6}$	*b*
	DR-CALUX in recombinant H4IIE cells	EC5: 7.5×10^{-6}	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.9 \times 10^{-6}$	*a*
2,3,6,7-tetraCN	EROD: PLHC-1 fish hepatoma cells	$<2.2 \times 10^{-3}$	*b*
	EROD: H4IIE recombinant cells	$<3.5 \times 10^{-4}$	*b*
	DR-CALUX in recombinant H4IIE cells	EC5: 4.2×10^{-5} EC20: 4.4×10^{-5} EC50: 4.1×10^{-5}	*a*
1,2,3,4,6-pentaCN	DR-CALUX in recombinant H4IIE cells	EC5: 5.0×10^{-5} EC20: 1.0×10^{-4} EC50: 6.8×10^{-5}	*a*

Congener	Assay	Relative Potency	Ref
	Micro-EROD in H4IIEC3/T cells	EC5: $2.5x10^{-5}$ EC20: $5.3x10^{-5}$ EC50:$4.3x10^{-5}$	*a*
1,2,3,5,7-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-5}$	*b*
	EROD: H4IIE recombinant cells	$<3.9x10^{-6}$	*b*
	DR-CALUX in recombinant H4IIE cells	EC5: $<3.4x10^{-6}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.8x10^{-6}$	*a*
1,2,3,5,8-pentaCN	DR-CALUX in recombinant H4IIE cells	EC5: $<1.8x10^{-6}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<1.2x10^{-6}$	*a*
1,2,3,6,7-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-3}$	*b*
	EROD: H4IIE recombinant cells	$2.7x10^{-4}$ to $2.2x10^{-5}$	*b*
	Luciferase assay: H4IIE recombinant cells	$<6.4x10^{-4}$	*b*
	Luciferase assay: H4IIE-luc cells	$1.7x10^{-4}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: 0.0018 EC20: $9.6x10^{-4}$ EC50: $5.8x10^{-4}$	*a*
1,2,3,7,8-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-5}$	*b*
	EROD: H4IIE recombinant cells	$3.9x10^{-5}$ to $1.3x10^{-5}$	*b*
	Luciferase assay: H4IIE recombinant cells	$4.6x10^{-5}$ to $4.5x10^{-5}$	*b*
1,2,4,5,6-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-6}$	*b*
	EROD: H4IIE recombinant cells	$7.8x10^{-6}$ to $3.1x10^{-7}$	*b*
	Luciferase assay: H4IIE recombinant cells	$8.1x10^{-6}$ to $1.5x10^{-6}$	*b*
1,2,4,6,7-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-6}$	*b*
	EROD: H4IIE recombinant cells	$<3.9x10^{-7}$	*b*
	Luciferase assay: H4IIE recombinant cells	$2.6x10^{-5}$ to $3.6x10^{-7}$	*b*
1,2,4,6,8-pentaCN	EROD: PLHC-1 fish hepatoma cells	$<2.5x10^{-5}$	*b*
	EROD: H4IIE recombinant cells	$<3.9x10^{-7}$	*b*
1,2,3,4,6,7-hexaCN	EROD: PLHC-1 fish hepatoma cells	$<2.8x10^{-4}$	*b*
	EROD: H4IIE recombinant cells	$1.5x10^{-3}$ to $2.6x10^{-4}$	*b*

Congener	Assay	Relative Potency	Ref
	Luciferase assay: H4IIE recombinant cells	$2.2x10^{-3}$ to $3.0x10^{-3}$	*b*
	Luciferase assay: H4IIE-luc cells	$4.0x10^{-3}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: 0.0014 EC20: 0.0017 EC50: 0.0012	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: 0.00058 EC20: 0.00053 EC50: 0.00054	*a*
1,2,3,5,6,7-hexaCN	EROD: PLHC-1 fish hepatoma cells	$<2.8x10^{-5}$	*b*
	EROD: H4IIE recombinant cells	$3.1x10^{-4}$ to $2.7x10^{-4}$	*b*
	EROD: H4IIE cells	0.002	*d*
	AHH: H4IIE cells	0.003	*d*
	Luciferase assay: H4IIE-luc cells	$1.0x10^{-3}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: $3.8x10^{-4}$ EC20: $6.1x10^{-4}$ EC50: $4.8x10^{-4}$	*a*
50:50 mix of 1,2,3,4,6,7-hexaCN & 1,2,3,5,6,7-hexaCN	Luciferase assay: H4IIE-luc cells	$1.3x10^{-3}$	*c*
1,2,3,5,6,8-hexaCN	EROD: PLHC-1 fish hepatoma cells	$<2.8x10^{-3}$	*b*
	EROD: H4IIE recombinant cells	$<4.4x10^{-4}$	*b*
	Luciferase assay: H4IIE-luc cells	$1.5x10^{-4}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: $2.8x10^{-4}$ EC20: $4.8x10^{-4}$ EC50: $4.9x10^{-4}$	*a*
1,2,3,5,7,8-hexaCN	DR-CALUX in recombinant H4IIE cells	EC5: $7.2x10^{-5}$ EC20: $1.6x10^{-4}$ EC50: $1.1x10^{-4}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $2.2x10^{-5}$ EC20: $1.4x10^{-5}$ EC50: $6.4x10^{-6}$	*a*
1,2,3,6,7,8-hexaCN	EROD: PLHC-1 fish hepatoma cells	$<2.8x10^{-4}$	*b*
	EROD: H4IIE recombinant cells	$1.7x10^{-3}$ to $2.6x10^{-3}$	*b*
	Luciferase assay: H4IIE recombinant cells	$2.2x10^{-2}$ to $4.5x10^{-3}$	*b*
	Luciferase assay: H4IIE-luc cells	$5.9x10^{-4}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: 0.0097 EC20: 0.0095 EC50: 0.0028	*a*
1,2,4,5,6,8-hexaCN	EROD: H4IIE cells	0.000007	*d*
	AHH: H4IIE cells	0.000007	*d*

Congener	Assay	Relative Potency	Ref
	DR-CALUX in recombinant H4IIE cells	EC5: $<1.1x10^{-6}$	*a*
1,2,4,5,7,8-hexaCN	DR-CALUX in recombinant H4IIE cells	EC5: $4.5x10^{-5}$ EC20: $9.0x10^{-5}$ EC50: $6.0x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $7.1x10^{-6}$	*a*
HexaCN – B1	EROD: H4IIE cells	0.00002	*d*
	AHH: H4IIE cells	0.00002	*d*
HexaCN – C1	EROD: H4IIE cells	0.002	*d*
	AHH: H4IIE cells	0.001	*d*
HexaCN – E1	EROD: H4IIE cells	0.002	*d*
	AHH: H4IIE cells	0.002	*d*
1,2,3,4,5,6,7-heptaCN	EROD: PLHC-1 fish hepatoma cells	$<3.1x10^{-3}$	*b*
	EROD: H4IIE recombinant cells	$3.8x10^{-4}$ to $5.6x10^{-4}$	*b*
	Luciferase assay: H4IIE recombinant cells	$1.3x10^{-3}$ to $3.8x10^{-4}$	*b*
	EROD: H4IIE cells	0.003	*d*
	AHH: H4IIE cells	0.003	*d*
	Luciferase assay: H4IIE-luc cells	$1.0x10^{-3}$	*c*
	DR-CALUX in recombinant H4IIE cells	EC5: $3.7x10^{-4}$ EC20: $5.9x10^{-4}$ EC50: $5.2x10^{-4}$	*a*
1,2,3,4,5,6,8-heptaCN	DR-CALUX in recombinant H4IIE cells	EC5: $4.1x10^{-6}$	*a*
OctaCN	DR-CALUX in recombinant H4IIE cells	EC5: $1.0x10^{-5}$	*a*
	Micro-EROD in H4IIEC3/T cells	EC5: $<4.3x10^{-6}$	*a*
Halowaxes			
Halowax 1001	EROD: PLHC-1 fish hepatoma cells	$<2.7x10^{-4}$	*e*
	EROD: H4IIE recombinant cells	$<4.2x10^{-5}$	*e*
	Luciferase assay: H4IIE recombinant cells	$<6.9x10^{-5}$	*e*
	EROD: H4IIE wild type cells	$<5.8x10^{-5}$	*e*
Halowax 1013	EROD: PLHC-1 fish hepatoma cells	$<2.7x10^{-6}$	*e*
	EROD: H4IIE recombinant cells	$7.0x10^{-7}$ to $7.5x10^{-12}$	*e*
	Luciferase assay: H4IIE recombinant cells	$7.7x10^{-6}$ to $6.2x10^{-8}$	*e*
	EROD: H4IIE wild type cells	$1.2x10^{-6}$ to $8.2x10^{-12}$	*e*
	Luciferase assay: H4IIE-luc cells	$1.8x10^{-6}$	*c*
Halowax 1014	EROD: PLHC-1 fish hepatoma cells	$<2.7x10^{-6}$	*e*
	EROD: H4IIE recombinant cells	$3.2x10^{-5}$ to $4.1x10^{-7}$	*e*

Congener	Assay	Relative Potency	Ref
	Luciferase assay: H4IIE recombinant cells	$6.7x10^{-6}$ to $6.5x10^{-7}$	*e*
	EROD: H4IIE wild type cells	$3.1x10^{-5}$ to $3.6x10^{-7}$	*e*
	Luciferase assay: H4IIE-luc cells	$3.8x10^{-5}$	*c*
Halowax 1051	EROD: PLHC-1 fish hepatoma cells	$<2.4x10^{-5}$	*e*
	EROD: H4IIE recombinant cells	$1.2x10^{-5}$ to $4.6x10^{-6}$	*e*
	Luciferase assay: H4IIE recombinant cells	$6.4x10^{-5}$ to $1.4x10^{-5}$	*e*
	EROD: H4IIE wild type cells	$1.6x10^{-4}$ to $2.8x10^{-5}$	*e*
	Luciferase assay: H4IIE-luc cells	$8.9x10^{-3}$	*c*

a: Behnisch *et al.*, 2003: REP values were either the ratio of EC(x)test:EC(x)TCDD or for EC5 interpolation of response induced by test compound onto TCDD dose response curve.
b: Villeneuve *et al.*, 2000: REP values range of estimates from multiple point estimates over response range from 20-80% TCDD max and extrapolation was used where maximum response was less than 80%. Where <x values are given, maximum concentration was divided by the EC50 of the TCDD standard.
c: Blankenship *et al.*, 2000: Relative potency at EC50 of TCDD.
d: Hanberg *et al.*, 1990: Estimation determined by comparison of activities with linear part of dose response curve for TCDD. Ratios calculated from amounts causing the same effects on enzyme induction or receptor binding.
e: Villeneuve *et al.*, 2001: REP values determined as is Villeneuve *et al.* (2000).

74. Table 1 summarises all the available REP values derived experimentally from a number of different assays and it should be noted that the derivation used differed between the papers. The Jiang *et al.* (2007) survey used the REP values determined by Blankenship *et al.* (2000) and Villeneuve *et al.* (2000) to derive the TEQ intake from PCNs. In the draft FSIS (Annex A), the Hanberg *et al.* (1990) and Blankenship *et al.* (2000) EROD or luciferase assay values were used to determine the TEQ contribution from the different foodstuffs analysed. Where more than 1 value was available, the worst case was used for the TEQ calculation.

Other Risk Assessments

International Programme on Chemical Safety: Concise International Chemical Assessment Document 34 – Chlorinated naphthalenes (2001)

75. This review concluded that PCNs should be included in the development of TEFs and although there are data gaps, due to strong relationship with TCDD, PCNs should be treated analogously concerning their toxicity. It was noted that confident risk characterisation could not be performed, however exposure should be minimised as much as possible as some effects, e.g. on endocrine function, have been shown to occur at very low doses, citing the Omura *et al.* (2000) study (IPCS, 2001).

US Environmental Protection Agency: Environmental Hazard Assessment (1975)

76. In 1975, the United States Environmental Protection Agency (US EPA) carried out an environmental hazard assessment of chlorinated naphthalenes. They

concluded that the hazard warranted a moderate level of concern and a number of recommendations were made. These included determining the environmental persistence of higher chlorinated congeners, monitoring in areas close to where discharges could occur and determination of the metabolic fate of these compounds with respect to epoxide formation due to concern over carcinogenic potential (USEPA, 1975).

US Environmental Protection Agency: Water Quality Criteria (1980)

77. The US EPA in 1980 reviewed the available data on chlorinated naphthalenes with a view to determining Water Quality Criteria. The lack of a no effect level for the congeners studied in repeat dose oral and inhalation studies was a significant hindrance, along with the differences in response between routes of exposure. It was noted that much of the data were produced to study the nature of the response in a variety of animal species rather than determining a dose-response relationship. Therefore the US EPA concluded that insufficient data were available to develop rational water quality criteria for chlorinated naphthalenes. It was noted that their chemical and physical properties suggested that persistence in the environment could be similar to that of PCBs (USEPA, 1980).

Results of the FSA investigation (Annex A)

78. Some or all of the PCN congeners were found in all the samples analysed, and the highest concentrations and highest frequencies of detection were in the oily fish samples. PCN 52 (1,2,3,5,7-pentaCN) was always the most abundant congener in fish, a finding consistent with the Llobet *et al.* (2007) survey in Spain, but in other foods it was not as dominant. The higher concentrations in fish are consistent with the Falandysz (2003) review of PCNs in food. The main dioxin-like congeners were PCNs 66/67 (1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN). When expressed as TEQs, PCNs 66/67 made the largest contribution in fish (around 70%). PCN 75 (octaCN) was detected only occasionally. In meat, offal and dairy products PCNs 66/67 and 73 (1,2,3,4,5,6,7-heptaCN) were detected more frequently. Total TEQ concentrations were in the range 0.0002-0.02 ng TEQ/kg fresh weight.

79. Exposure to the UK population has not been determined as part of this investigation, as the small number of samples taken means that the concentrations are unlikely to be representative.

Chlorinated Paraffins

80. The sections below provide an update on the available data since the completion of the European Risk Assessment Reports in 1999 for SCCPs and the 2004 draft human health report on MCCPs and should therefore be considered in conjunction with these.

Chemistry

81. CPs are chlorinated linear hydrocarbons with between 10 and 30 carbon atoms containing varying numbers of chlorine atoms, with a maximum of one chlorine atom per carbon. They are manufactured by the chlorination of liquid paraffin (WHO, 1996).

Absorption, Distribution, Metabolism, Excretion

82. 6 groups of 1 day old Lohmann meat broiler chickens were fed diets containing SCCPs with 60% chlorine content at 0 (n=48), 2 (n=8), 20 (n=8), 45 (n=8), 70 (n=8) or 100 (n=48) mg/kg feed for 31 days. There was a linear relationship between concentration in feed and those in the tisses with highest amounts in the abdominal fat. Less than 5% of the ingested CPs remained in the tissues excluding the head, gut, feet and feathers. The authors attributed remaining amounts to other tissues and droppings (Ueberschär and Matthes, 2004).

83. 6 groups of laying Lohmann selected Leghorn hens aged 24 weeks were fed diets containing SCCPs with 60% chlorine content at 0 (n=21), 2 (n=9), 20 (n=9), 45 (n=9), 70 (n=9) or 100 (n=36) mg/kg feed for 8 weeks (equivalent to 0, 0.25, 2.5, 5.6, 8.8 or 12.5 mg/kg b.w./day). 10 eggs from different birds were collected on 9 occasions during the experiment for analysis of CP content. 9 animals from each group were terminated when dosing finished and tissue samples were assessed for CP content. Concentrations of the CPs in feed had a linear relationship with the concentrations found in the tissues and blood but not with the bile fluid. Highest concentrations were found in the abdominal fat, yolk and liver. No residues were found in egg albumen (Ueberschär *et al.*, 2007).

84. From this study, 24 of 36 animals which had received 100 mg/kg feed (12.5 mg/kg b.w./day) were kept for a withdrawal study. 3 animals were killed on days 1, 2, 4, 8, 14, 21, 28 and 42 after cessation of dosing with 3 animals from the control group killed on days 14, 28 and 42. Yolk and albumen were sampled at various time points from 6 to 10 eggs. Concentrations in yolk showed a rapid drop following cessation of dosing and then a slow phase of elimination. All tissues showed a rapid and a slow phase of decrease in tissue concentrations with the rapid phase being particularly distinct for the yolk, blood and liver. Rapid phase half-life in blood was approximately 42 mins and liver, kidney and leg muscle was 4-10 mins whereas yolk was approximately 2.9 days. Terminal half-lives in all tissues were approximately 20-30 days. Authors suggested this to be related to equilibrium partitioning between blood and slowly perfused adipose tissue. About 30% of the ingested CPs were excreted in the urine and faeces and 1.5% was estimated for the yolk, with approximately 1% remaining in the body. The authors suggested that the remainder may be in tissues that were not analysed or result from excretion from an unmeasured route or errors in estimation (Ueberschär *et al.*, 2007).

Toxicity

85. This section summarises the recent mammalian and avian studies conducted on chlorinated paraffins.

86. As part of an experiment looking at the effects of various chlorinated compounds, singly and as mixtures, on thyroid hormone levels, female Sprague Dawley rats were given Witaclor 171P (an SCCP with 71% chlorine content) orally once a day for 14 days. Animals were sacrificed 24 hours after the final dose. Blood samples were taken, liver and thyroid glands were weighed and a liver sample taken for enzyme analysis. Results showed no differences in the response seen in Witaclor treated animals or controls. Some changes in response were seen when Witaclor was combined with DE-47 (a polybrominated diphenyl ether congener) and/or Aroclor 1254 (a technical preparation of polychlorinated biphenyls) (Hallgren and Darnerud, 2002).

87. 6 groups of 1 day old Lohmann meat broiler chickens were fed diets containing SCCPs with 60% chlorine content at 0 (n=48), 2 (n=8), 20 (n=8), 45 (n=8), 70 (n=8) or 100 (n=48) mg/kg feed for 31 days. There was no influence of CPs on feed intake, live weight gain or feed to gain ratio. Macroscopic examination revealed no lesions or pathological anomalies of the organs. No relative weight ratios were significantly different in treated groups compared to controls except the spleen in the highest dose group. Lower live weight (control 1490 g ± 141, high dose 1420 g ± 171, p=0.04) and a higher relative spleen weight (control 0.090% ± 0.019, high dose 0.10% ± 0.04, p=0.03) was found (Ueberschär and Matthes, 2004).

88. 6 groups of laying Lohmann selected Leghorn hens aged 24 weeks were fed diets containing SCCPs with 60% chlorine content at 0 (n=21), 2 (n=9), 20 (n=9), 45 (n=9), 70 (n=9) or 100 (n=36) mg/kg feed for 8 weeks (equivalent to 0, 0.25, 2.5, 5.6, 8.8 or 12.5 mg/kg b.w./day). Laying performance of the control and high dose groups were assessed. At termination when dosing ceased, organ weights were assessed in nine animals per group. There were no indications of the CPs affecting the laying performance of any of the treated birds. Absolute and relative weight of the pancreas in the high dose group was decreased with the relative weight being significantly decreased. Neither live weight nor other organs were affected by treatment (Ueberschär *et al.*, 2007).

Estimates of dietary exposure

89. A market basket study has been carried out in Japan which showed that the highest concentrations are found in fats (140 ng/g), followed by fish and shellfish (16-18 ng/g) and meat (7.0 ng/g). The milk category comprising milk, cream and yoghurt had the lowest concentration. Total daily intakes were estimated for different age groups with highest exposure occurring in 1 year old females at 680 ng/kg b.w./day which authors considered was not a risk to human health (Iino *et al.*, 2005).

90. One study has analysed human milk samples for SCCPs and MCCPs. Samples obtained from Lancaster (n=5) and London (n=20) in 2001 and 2002. There was no evidence of significant differences between the two sites. The median

concentration of SCCPs was 180 ng/g fat (range 49 to 820 ng/g fat) and MCCPs 21 ng/g fat (range 6.2 to 320 ng/g fat). The authors note that there is a substantial difference in transfer efficiency between SCCPs and MCCPs as MCCPs are present at higher concentrations in the environment and more has been produced than SCCPs but the concentration in milk is much lower (Thomas *et al.*, 2006).

Other Risk Assessments

European Union Risk Assessment Report for SCCPs produced by the European Chemicals Bureau (1999)

91. SCCPs show low acute toxicity to animals, minimal skin and mild eye irritation and are not mutagenic. Kidney adenomas have been observed in male rats and could not be clearly demonstrated to be male rat specific, however due to lack of genotoxic effect, it is unlikely that there will be human consequences below the level causing chronic toxicity (UK HSE, 1999).

92. For consumers of products containing SCCPs, the margin of exposure between calculated exposures (0.03 mg/kg b.w./day for metal working fluids and 0.02 mg/kg b.w./day for leather and textile use) and the NOAELs for general toxicity (100 mg/kg b.w/day), kidney carcinogenicity (100 mg/kg b.w./day) and developmental effects (500 mg/kg b.w./day) from repeat dose studies are over 3 orders of magnitude. Similarly for indirect human exposure resulting from releases into the environment, the margin of exposure was three orders of magnitude for repeat dose toxicity and carcinogenicity and six orders of magnitude for developmental effects. These margins were derived from the consideration that food and to a lesser extent drinking water would be the main sources of exposure and a worst case estimate of exposure from the environment of 0.020 mg/kg b.w./day (UK HSE, 1999).

93. The conclusions of this assessment were that there was at the time of writing no need for further information and/or testing or for risk reduction measures beyond those which are being applied already with respect to human health through occupational exposure, consumer exposure, and exposure via environmental routes (UK HSE, 1999).

EC Scientific Committee on Toxicity, Ecotoxicity and the Environment Opinion on SCCPs (2002)

94. The EC Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) reviewed the new data available since the ECB Risk Assessment Report. It concluded that the new data did not alter the conclusions drawn in report (CSTEE, 2002).

Draft European Union Risk Assessment Report for human health effects of MCCPs produced by the European Chemicals Bureau (2004)

95. MCCPs show low acute oral toxicity and only slight irritation on single dermal exposure which is more pronounced with repeated exposure. Only slight eye irritation has been observed. Following repeated exposure, of the liver effects observed, only

single cell necrosis was considered to be of relevance to humans. A NOAEL of 0.4 mg/kg b.w./day for repeated dose toxicity was identified on the basis of inner medullary tubular dilatation in female rat kidney and for thyroid changes. Reproductive studies showed internal haemorrhaging in rat pups before weaning which was determined to be mediated through the breast milk. A NOAEL of 8 mg/kg b.w./day maternal dose was identified from these studies. In general it was considered that in the absence of data on MCCPs, it was valid to read across from SCCPs. Therefore the carcinogenic potential of MCCPs should be considered similar to that of SCCPs (UK HSE, 2004).

96. The conclusions of this assessment were that there was at the time of writing no need for further information and/or testing and for risk reduction measures beyond those which were being applied already with respect to human health via most occupational exposures, consumer exposure and exposure through environmental routes except for infants exposed through breast or cow's milk. There was a need for further information and/or testing for exposures to workers during compounding of PVC and metal working fluids use and for infants exposed via breast or cow's milk. In the case of infants, there was a five order of magnitude difference between estimated uptake of infants (60 x 10^{-5} mg/kg b.w./day for breast milk and 29 x10^{-5} mg/kg b.w./day for cows milk) and levels at which adverse effects have been identified in animals (estimated pup intake 93 mg/kg b.w./day). However, there were uncertainties in the exposure assessment, which could be improved with information on levels in human breast and cow's milk, and the significance of a haemorrhaging effect observed in pups leads to concern for human infants (UK HSE, 2004).

97. It was concluded that for workers exposed during paint spraying there is a need for limiting the risks as the margin between exposure and effects in repeat dose toxicity and lactation were unacceptably low. It was proposed that MCCPs be labelled with the risk phrases R64 (may cause harm to breast-fed babies) and R66 (repeated exposure may cause skin dryness or cracking). The need for limiting risk was also concluded for regional exposure with respect to carcinogenicity (UK HSE, 2004).

<u>World Health Organization (1996) monograph on Chlorinated Paraffins</u>

98. The World Health Organization (WHO) considered that the principal source of exposure to the general population is food. CPs are bioaccumulative and persistent. SCCPs were determined to be carcinogenic in rats and mice, no data were available on MCCPs and LCCPs had limited evidence of carcinogenicity. The available data indicated that the carcinogenic effects are not mediated via direct interaction with DNA (WHO, 1996).

99. Tolerable daily intakes (TDIs) for non-neoplastic effects of SCCPs, MCCPs and LCCPs were derived. For SCCPs, the lowest reported NOAEL of 10 mg/kg b.w./day for increases in liver and kidney weight and hypertrophy of the liver and thyroid from a 13 week study in rats was used with a standard uncertainty factor of 100 to derive a TDI of 100 µg/kg b.w./day. Similarly for MCCPs, a TDI of 100 µg/kg b.w./day was derived from a NOAEL of 10 mg/kg b.w./day for increases in liver and kidney weights in a 13 week study in rats with a standard uncertainty factor of 100. For LCCPs, the TDI was derived from a LOAEL of 100 mg/kg b.w./day for increased

liver weight and multifocal granulomatous hepatitis in female rats a long term study with an uncertainty factor of 1000 (10 for interspecies variability, 10 for interindividual variability and 10 for use of a LOAEL rather than a NOAEL) (WHO, 1996).

100. In addition, a maximum daily dose on the basis of neoplastic effects of SCCPs was derived. Multistage modelling of the hepatocellular adenomas and carcinomas in male mice, the tumours with the highest incidence, estimated a dose of 11 mg/kg b.w./day would be associated with a 5% increase in the tumour. An uncertainty factor of 1000 for a non-genotoxic carcinogen was used to derive the recommended maximum daily dose of 11 µg/kg b.w./day on the basis of neoplastic effects (WHO, 1996).

101. Recommendations for future research included developing selective and sensitive methods of analysis to provide reliable data on CPs in foodstuffs and human tissues, studying the influence of chain length and degree of chlorination on toxicodynamics and toxicokinetics, investigating toxicokinetics and half-lives of CPs in mammals and studying perinatal toxicity (WHO, 1996).

International Agency for Research on Cancer (1990) monograph on Chlorinated Paraffins

102. IARC considered there to be sufficient evidence for carcinogenicity of a SCCP (C12, 60% Cl) in experimental animals and limited evidence for carcinogenicity of a LCCP (C23, 43% Cl) in experimental animals. No human data on carcinogenicity were available. SCCPs of an average length of 12 carbon atons and with an average degree of chlorination of 60% were considered to be Group 2B, possibly carcinogenic to humans (IARC, 1990).

Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Priority Existing Chemical Assessment Report on SCCPs (2001)

103. In their analysis the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) determined that there was significant absorption of SCCPs by the oral but not the dermal route. Distribution was mainly to tissues with high cell turnover or high metabolic activity and SCCPs are metabolised by CYPs to CO_2 and excreted via urine, exhaled air or faeces. The kinetics are affected by degree of chlorination. SCCPs show low acute toxicity to animals and there is no evidence of skin irritation or sensitisation in humans. Repeated dose toxicity and carcinogenicity studies in animals show liver, kidney and thyroid effects though mechanistic information suggests liver and thyroid effects are not relevant to humans. The mechanism for kidney tumours is unclear and NICNAS support the EU classification of SCCPs as category 3 carcinogens. Overall evidence suggests that SCCPs are not mutagenic (NICNAS, 2001).

104. NICNAS considered that exposure of the general public to SCCPs through use in products is not a significant hazard to public health, however exposure through food is possible and releases to the environment should be kept to a minimum. Data gaps were identified with respect to effects of different chain lengths and degree of chlorination to toxicological endpoints and SCCP levels in food (NICNAS, 2001).

The Organisation for Economic Co-operation and Development SIDS Initial Assessment Report on SCCPs

105. This review on behalf of the Organisation for Economic Co-operation and Development (OECD) found limited toxicokinetic information but determined that there is approximately 60% absorption via oral route. Acute and skin irritation studies indicated independence of chain length and degree of chlorination and showed low acute toxicity and at most, minimal skin and mild eye irritation were reported in animals. Principal signs of toxicity in animals are effects in liver and thyroid however mechanistic data indicated that these were not relevant to human health. NOAELs of 100 mg/kg b.w./day in rats and 1000 mg/kg b.w./day in mice for decreased body weight gain and increased kidney weights were identified. Developmental effects were observed in rats but only at maternally toxic doses (OECD).

106. With respect to mutagenicity and carcinogenicity, overall the evidence indicated that SCCPs are not mutagenic. Carcinogenicity studies showed dose related increases in adenomas and carcinomas in the liver, thyroid and kidney. It was concluded that peroxisome proliferation was likely to be the mechanism underlying the liver tumours and long term stimulation of the thyroid was likely to be a consequence of the liver effects. Kidney adenomas were seen exclusively in male rats and while it was thought that this may be due to a male rat specific event, this could not be clearly demonstrated. It was therefore suggested that there is the possibility of kidney adenomas being relevant to human health. In the absence of genotoxicity, it was considered unlikely that kidney tumours will occur below the level causing chronic toxicity in this organ. Therefore a NOAEL of 100 mg/kg b.w./day was appropriate to assess kidney carcinogenicity (OECD).

Results of the FSA survey (Annex A)

107. Short chain (SCCPs) and medium chain chlorinated paraffins (MCCPs) were detected less frequently than PCNs, but most often in fish at low concentrations (<1.0-6.0 and <1.0-39 µg/kg). In the fresh eels sample, which contained the highest concentrations found in the project, it is likely that LCCPs were also present. SCCPs and/or MCCPs were also detected in the butter, pork sausage, bread, fruit and one each of the beef and lamb samples, but very seldom or not at all in the milk products, eggs and poultry samples. The highest concentrations were in the cod liver oil sample (25 and 72 µg/kg). This is consistent with concentrations found in fish elsewhere. Where detected, MCCPs were usually more abundant than SCCPs. The SCCPs results are similar to those found in the Japanese market basket survey (Iino *et al.*, 2005).

108. Exposure to the UK population has not been determined as part of this investigation, as the small number of samples taken means that the concentrations are unlikely to be representative.

Questions on which the views of the Committee are sought

109. Members are invited to consider the following questions and to raise any other matters that arise from the draft Food Surveillance Information Sheet and the data provided in this paper.

i). Does the Committee consider the available data are sufficient to propose a TEF approach for PCNs for inclusion in the TEQ for dioxins and dioxin-like PCBs? If so what TEFs should be applied?

ii). Based on the available data would it be more appropriate to establish health based guidance values or adopt a Margin of Exposure approach to assessment of health risks of PCNs or CPs? What NOAELs and uncertainty factors should be applied?

iii). Do the results presented in the draft Food Surveillance Information Sheet raise concerns for UK consumers?

iv). Does the Committee have recommendations for any further work?

Secretariat
January 2009

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Food Surveillance Information Sheet: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food

Draft Food Surveillance Information Sheet.

A draft of this document has been provided to the Committee. Once finalised incorporating the COT conclusions, it will be available from the Food Standards Agency website www.food.gov.uk

Secretariat
January 2009

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Food Surveillance Information Sheet: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food

European Union Risk Assessment Report: Alkanes, C_{10-13}, chloro (1999)

This is the European Union risk assessment report, prepared by the UK Health and Safety Executive, summarising the available data on short chain chlorinated paraffins.

This document is publicly available from the European Commission website at: http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/sccpreport010.pdf

Secretariat
January 2009

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Food Surveillance Information Sheet: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food

Draft European Union Risk Assessment Report: Alkanes, C_{10-13}, chloro (2004)

This is the draft European Union risk assessment report, prepared by the UK Health and Safety Executive, summarising the available data on medium chain chlorinated paraffins.

Once finalised, this document will be publicly available from the European Commission website at: http://ecb.jrc.ec.europa.eu/

Secretariat
January 2009

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Food Surveillance Information Sheet: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food

Search Strategy

This Annex presents the search strategy used to obtain the papers for this discussion document.

Secretariat
January 2009

General Search September 2006

Databases interrogated –

- Food Science and Technology Abstracts (FSTA)
- FoodlineWeb
- British Library Direct

Search Terms – (polychlorinated-napthalene* OR chlorinated paraffin* OR chloroparaffin*) AND (toxicolog* OR risk assessment OR food* OR TEF*)
Search Dates (From/To) – No parameters set

Exclusion Criteria – Abstracts where reviewed and papers excluded if they:

- Focussed on temporal trends – 1 paper rejected
- Were exposure assessments or contained mainly survey data – 1 paper rejected
- Concentrated on sampling, measurement, analysis and screening methods – 4 papers rejected
- Were not concerned with toxicity – 12 papers rejected
- Focused on eco-toxicity – 1 paper rejected

Number of Abstracts Identified – 27
Number of Papers Requested – 8

Updated Search October 2008

Databases interrogated –

- PubMed

Search Terms – (polychlorinated naphthalene* OR chlorinated paraffin* OR chloroparaffin*) AND (toxicolog* OR risk assessment OR food* OR TEF*)
Search Dates (From/To) – No parameters set

Exclusion Criteria – Abstracts where reviewed and papers excluded if they:

- Were literature reviews of existing papers – 6 papers rejected
- Focussed on temporal trends – 3 papers rejected
- Were exposure assessments or contained mainly survey data – 13 papers rejected
- Focussed on environmental contamination and/or bioaccumulation – 25 papers rejected
- Concentrated on sampling, measurement, analysis and screening methods – 4 papers rejected
- Did not consider Polychlorinated Naphthalene's (PCN's) or Chlorinated Paraffin's (CP's) in any detail – 3 papers rejected

Number of Abstracts Identified – 82
Number of Papers Requested – 28

Further Updated Search October 2008

Databases interrogated –

- PubMed

Search Terms – (Chloro* Naphthalene OR Polychloro* Naphthalene OR Chlorinated Naphthalene OR Chloro* Alkane) AND (toxicolog* OR risk assessment OR food* OR TEF*)
Search Dates (From/To) – No parameters set

Exclusion Criteria – Abstracts where reviewed and papers excluded if they:

- Were literature reviews of existing papers – 1 paper rejected
- Focussed on temporal trends – 0 papers rejected
- Were exposure assessments or contained mainly survey data – 0 papers rejected
- Focussed on environmental contamination and/or bioaccumulation – 0 papers rejected
- Concentrated on sampling, measurement, analysis and screening methods – 1 paper rejected
- Did not consider Polychlorinated Naphthalene's (PCN's) or Chlorinated Paraffin's (CP's) in any detail – 31 papers rejected
- Were not concerned with toxicity – 3 papers rejected

Number of Abstracts Identified – 37
Number of Papers Requested – 1

PCNs – General and Updated Search October 2008

Databases interrogated –

- ISI Web of Knowledge Current Contents database
- Food Science and Technology Abstracts (FSTA)
- FoodlineWeb
- IngentaConnect
- British Library Direct Plus

Search Terms – Polychlorinated-naphthalene* AND (toxicolog* OR risk -assessment OR food* OR TEF*)
Search Dates (From/To) – From Jan 2000 to Oct 2008

Exclusion Criteria – Abstracts where reviewed and papers excluded if they:

- Were literature reviews of existing papers – 8 paper rejected
- Focussed on temporal trends – 1 papers rejected
- Were exposure assessments or contained mainly survey data – 3 papers rejected
- Focussed on environmental contamination and/or bioaccumulation – 33 papers rejected
- Concentrated on sampling, measurement, analysis and screening methods – 16 paper rejected

- Did not consider Polychlorinated Naphthalene's (PCN's) or Chlorinated Paraffin's (CP's) in any detail – 4 papers rejected
- Were not concerned with toxicity – 6 papers rejected
- Were duplicated papers or ones already held – 51 papers rejected

Number of Abstracts Identified – 123
Number of Papers Requested – 1

CPs – General and Updated Search October 2008

Databases interrogated –

- ISI Web of Knowledge Current Contents database
- Food Science and Technology Abstracts (FSTA)
- FoodlineWeb
- IngentaConnect
- British Library Direct Plus

Search Terms – (chlorinated-paraffin* OR chloroparaffin*) AND (toxicolog* OR risk-assessment OR food* OR TEF*)
Search Dates (From/To) – From Sept 2006 to Oct 2008

Exclusion Criteria – Abstracts where reviewed and papers excluded if they:

- Were literature reviews of existing papers – 6 paper rejected
- Focussed on temporal trends – 1 papers rejected
- Were exposure assessments or contained mainly survey data – 0 papers rejected
- Focussed on environmental contamination and/or bioaccumulation – 10 papers rejected
- Concentrated on sampling, measurement, analysis and screening methods – 3 paper rejected
- Did not consider Polychlorinated Naphthalene's (PCN's) or Chlorinated Paraffin's (CP's) in any detail – 0 papers rejected
- Were not concerned with toxicity – 11 papers rejected
- Were duplicated papers or ones already held – 14 papers rejected

Number of Abstracts/Papers Identified – 50
Number of Papers Requested – 5 which did not have abstracts and might therefore be excluded on review of the full paper.

Analysis of review papers

A number of review papers were identified during these searches. Any papers cited in relevant sections of these reviews but not identified by the searches were also obtained.