

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

First Draft Statement on Polychlorinated Naphthalenes in Food

Introduction

1. In February 2009, the Committee discussed paper TOX/2009/02 on polychlorinated naphthalenes and chlorinated paraffins in food and agreed that the issues should be considered separately.
2. The first draft statement on polychlorinated naphthalenes based on the discussions and conclusions at the February 2009 meeting is attached at Annex A.
3. The evidence presented at the February meeting, did not suggest that all the toxic effects of PCNs occur through interaction with the aryl hydrocarbon receptor (AhR), or what type of activity the PCNs have at the receptor. However, it would be reasonable to assume PCNs are less persistent than TCDD, and therefore summing concentrations in food on the basis of *in vitro* data on relative potency to TCDD would provide a highly conservative approach to cumulative risk assessment. It could not be assumed that all toxic effects of PCNs would be mediated via the AhR and the data on toxic effects other than AhR mediated dioxin-like activity would need further analysis before the Committee could comment on them. To this end, a table, which summarises relevant repeat dose studies, has been incorporated into the draft statement as Table 1.

Questions on which the views of the Committee are sought

4. Members are invited to consider the structure and content of the draft statement.

**Secretariat
March 2009**

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Note: This is a draft working paper for discussion. It does not reflect the final views of the Committee and should not be cited.

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First Draft Statement on Polychlorinated Naphthalenes in Food

Introduction

1. The Committee was asked for advice on possible risks associated with the results of a Food Standards Agency (FSA) investigation of polychlorinated naphthalenes (PCNs) in a number of foodstuffs.

Background

2. PCNs are a group of 75 congeners, with structures similar to those of polychlorinated dibenzo-p-dioxins and -furans (PCDDs and PCDFs). The PCN congeners contain between one and eight chlorine atoms bound to the naphthalene structure shown in Figure 1¹.

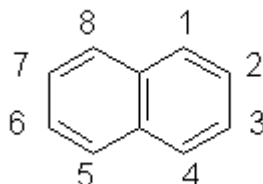


Figure 1: Naphthalene structure and the positions where chlorine atoms can potentially bind.

3. PCNs 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 (PCBs). 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 products¹.

4. In the 1930s and 1940s, PCNs were associated with chloracne and, in some instances, liver disease in exposed workers. Later they were shown to be causative agents of bovine hyperkeratosis, due to interference with the biotransformation of

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carotene to vitamin A, which affected cattle in a number of countries with great economic implications¹.

5. Resistance of PCNs against biotic degradation, and hence persistence in the environment, decreases with decreasing chlorine content and depends on the number of vicinal carbon atoms unsubstituted with chlorine². PCNs have been detected in fish and human milk in other countries. The FSA investigation was carried out as there were currently no UK data on PCNs in food. The papers used for this review were identified using the search strategy outlined in Annex A.

Kinetics

Absorption

6. From studies on the metabolism of PCNs, it can be concluded that mono- and dichloronaphthalenes (>80–90%) and tetrachloronaphthalenes (>45%) are well absorbed by the gastrointestinal tract. Higher chlorinated PCNs are less well absorbed, presumably very poorly absorbed¹. Rapid absorption was seen in four male Wistar rats given a single oral dose of 400 mg/kg radiolabelled 1,2-dichloronaphthalene with the highest blood level 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 level³.

Distribution

7. Groups of 3 male JCL:ICR mice were given a 1 mg single intraperitoneal (i.p.) dose of either 1,8-dichloronaphthalene, 2,7-dichloronaphthalene or octachloronaphthalene and sacrificed at intervals up to 28 days for investigation of the PCN tissue distribution. 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 was not consistent between the three congeners. 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, due to the lipophilic properties of PCNs. The half-life of 2,7-dichloronaphthalene was longer than that of 1,8-dichloronaphthalene in almost all tissues, and octachloronaphthalene had a longer biological half-life than the two dichloro- congeners suggesting that the position of the substituted chlorine and the number of chlorine atoms were influential⁴.

8. Ten male Wistar rats were given a single oral gavage dose of 400 mg/kg radiolabelled 1,2-dichloronaphthalene and sacrificed after 24 hours or 7 days for tissue distribution studies. Animals used in the absorption study described in para 6 provided 48 hour tissue distribution samples. After the first day, the liver and intestines contained the highest percentage of the total dose however by day 7, radioactivity was only detected in the skin and adipose tissue and corresponded to 0.05% of the total dose³.

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9. When a single dose of 10 mg/kg b.w. tritiated 1,2,3,4-tetra- and 1,2,3,4,5-pentachloronaphthalene was administered i.p. to male outbred Wistar rats the highest concentrations were found in fat, liver, kidney and adrenals. The highest concentrations in plasma and tissues were reached after 12 hours for the tetra- and 24 hours for the penta- congener⁵.

10. 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, and were sacrificed after 1, 10, 30 or 120 days for analysis of PCNs in the liver and intraperitoneal fat. On the first day after dosing, the PCN profile in the tissues was similar to that of the technical mixture although the relative levels of hepta- and octa- congeners were lower, which the authors suggested may be explained by lower absorption. A hexa- congener was dominant in the intraperitoneal fat from days 10 to 120, when this was the only congener detected and 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 of hexachloronaphthalene present. This animal was sacrificed after 30 days and one hexa- and one hepta- congener were identified⁶.

11. The distribution and metabolism of 1- and 2-chloronaphthalene has been studied in female Yorkshire pigs given an oil emulsion of the compounds by retrocarotid injection, 1 animal per compound (no control stated). Blood samples were obtained at intervals up to 6 hours when the pigs were sacrificed and total urine, bile, blood and organ samples were taken. The highest concentrations of the two PCNs in blood were measured at 10 minutes. After 6 hours the highest concentrations were in brain and kidney, although they were also detected in liver, lung, skeletal and back muscle and heart. In addition, 2-chloronaphthalene was detected at low levels in fat. Metabolites of both compounds were detected in liver and kidney⁷.

12. Samples of human 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. The liver generally contained higher levels than adipose tissue and a range of tetra- to hexa- congeners were found⁸.

Metabolism

13. Male Wistar rats were given a single oral dose of 3 different dichloronaphthalenes, with collection of urine for two days. 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 led 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 compounds⁹.

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14. Three rabbits were given a single 300 mg/kg dose of 2,6-dichloronaphthalene (route not stated) and four Sprague Dawley rats were similarly given a single 1 g/kg dose and urine was collected for a week. Pooled urine samples contained unchanged 2,6-dichloronaphthalene, a monochloronaphthol derivative and a dichloronaphthol derivative¹⁰.

15. Groups of 3 male albino rabbits were given a 1 g single dose of naphthalene, 1-chloronaphthalene, di-, tetra-, penta-, hepta- or octachloronaphthalene by gastric gavage. Twenty-four-hour urine samples were collected for 4 days and levels of creatinine, glucuronic acids, phenolic compounds, sulphur partitions and mercapturic acid were measured. Conjugate metabolites of penta-, hepta- and octachloronaphthalene were not observed in this study whereas 45% of the administered tetra- congener dose was 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. The authors suggest this indicates the penta-, hepta- and octa- congeners do not undergo this metabolism to these conjugates¹¹.

16. Pigs were administered 30 mg chloronaphthalenes/kg b.w. by retrocarotid injection in corn oil. After 5 hours animals were sacrificed and urine collected. 1-Chloronaphthalene produced 4-chloro-1-naphthol, and trace amounts of a dihydroxy compound. 2-chloronaphthalene was shown to be metabolised to 3-chloro-2-naphthol¹².

17. A female Yorkshire pig was given a single dose of 1-chloro-4-[²H]-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 was metabolised to 2,4-dichloronaphthol and 1,2,3,4-tetrachloronaphthalene was 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 administration¹³.

18. In the distribution and metabolism study described in para 11, 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, these metabolites were found in urine and bile⁷.

Elimination

19. Two rats with cannulated bile ducts were given 200 mg/kg radiolabelled 1,2-dichloronaphthalene by injection in the genital vein. Serial bile samples were collected for 72 hours after administration and analysed for radioactivity. Urine and

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faeces were 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 occurred. Analysis of the faeces indicated that the compound was unchanged whereas in the urine, the radioactivity was found as a glucuronide conjugate of a dihydrodiol³.

20. When single doses of 10 mg/kg b.w. tritiated 1,2,3,4-tetra- and 1,2,3,4,5-pentachloronaphthalene were administered i.p. to male Outbred Wistar rats 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 congeners⁵.

21. 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 octachloronaphthalene or its metabolites may be excreted in the milk¹⁴.

22. The half-lives of hexachloronaphthalene in rat liver and adipose tissue have been determined as 36 and 41 days respectively¹⁵. Human blood samples taken from the Yusho and Yu-cheng rice oil contamination incidents in 1968 and 1979 have been analysed for PCN content and the PCN half-life was estimated to be 1.5-2.4 years from 3 individuals from the Yu-cheng incident¹⁶. An analysis of human milk samples from Sweden collected between 1972 and 1992 showed a decrease to 16% of the 1972 level by 1992 indicating a half life of 8 years¹⁷.

Toxicity

23. The database on PCNs is very variable as studies have been carried out on different congeners or commercial mixtures 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 1930s and 1940s. Subsequently, PCNs were found to be the causative agents of bovine hyperkeratosis and numerous studies were carried out in cattle as a result, and investigations in sheep and pigs followed to determine the effects in these species.

24. 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)¹. It should also be noted that authors have

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commented that consideration of tests using technical mixtures should take into account the possibility of dioxin-like impurities, which may also cause an effect².

Acute toxicity

25. Three acute toxicity studies have been carried out. A 2008 study in rats used a tetra-hepta congener mixture and at all doses (250-1000 mg/kg b.w.), there was a loss in bodyweight and 72 hours after dosing with 500 and 1000 mg/kg b.w., an increase in relative liver mass. Liver and serum enzyme activities were determined leading the authors to suggest that the mechanisms of PCNs' toxic action is probably associated with oxidative stress induction due to changes in malondialdehyde¹⁸. In another study, individual congeners were administered to rabbits. 1 g hepta- and octa- congeners caused death of all 3 animals within 7 days and 1 g of penta- congeners caused death of 1 animal in this timeframe. In contrast, 1 g of chloronaphthalenes containing up to 4 chlorine atoms caused no apparent signs of toxicity¹¹. Single doses of penta- (22 mg/kg b.w.) or hexa- (11 mg/kg b.w.) congeners administered to calves in gelatine capsules caused hyperkeratosis, and at autopsy, gross and microscopic lesions of the liver and kidney were seen¹⁹.

Repeat dose toxicity

26. Table 1 provides a summary of the repeat dose and developmental studies using oral administration showing the most sensitive effects, mainly where more than one dose group has been considered in laboratory species, or for the farm species where the doses are at or below the doses given in laboratory animals, where the group size per dose was greater than 1 and the dose given was consistent throughout the study.

Table 1: Summary of selected repeat dose and developmental studies on PCNs.

Type of study	Substance and dose	Most sensitive endpoint	LOAEL	NOAEL
Subchronic toxicity				
7, 14 or 21 day oral gavage in male Wistar rats (n = 5 per group for each time point) ²⁰	Mixture of 54% tetra-, 8% penta-, 23% hexa- and 14% heptaCN – less than 0.1 pg dioxins and furans per 100 µg sample 0, 1, 10 or 100 mg/kg b.w./day	Increase in hepatic malondialdehyde after 21 days Also not dose related: Statistically significant increase in relative liver weight at all time points Increase in total CYPs in liver and statistically significant increase in CYP1A activity at all time points	1 mg/kg b.w./day	-
22 day feeding study in Holtzman rats (n = 6 per group) ²¹	Halowax 1051 with approximately 90% octa- and 10% heptaCN 0, 50, 200 or 500 mg/kg b.w./day ^b (0.05, 0.2 or 0.5% in the diet)	Reduction in stored vitamin A in liver and kidney	50 mg/kg b.w./day ^b (0.05% in diet)	-
Rat metabolism study by oral gavage for up to 5 weeks (group size not specified) ²²	Mixture consisting mainly of penta- and hexaCN 6.7, 33, 67 or 100 mg/kg b.w./day ^a (1, 5, 10 or 15 mg/ day to 150 g rat)	Death within 3 weeks	100 mg/kg b.w./day ^a (15 mg/day to 150 g rat)	67 mg/kg b.w./day ^a (10 mg/day to 150 g rat)

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Type of study	Substance and dose	Most sensitive endpoint	LOAEL	NOAEL
8 week feeding study in Sprague Dawley rats (n = 3 to 4 per group) ²³	Commercial mixture of tetra-, penta- and hexaCN with 62% chlorine content 2.5, 5.0, 10.0 or 40.0 mg/kg b.w./day ^b (25, 50, 100 or 400 mg/kg feed)	Reduction in liver vitamin A content * total weight gain decreased in dose dependent manner but no indication of when this was statistically different from control	10 mg/kg b.w./day ^b (100 mg/kg feed)	5 mg/kg b.w./day ^b (50 mg/kg feed)
8 week feeding study in hamsters (n = 9) ²³	Commercial mixture of tetra-, penta- and hexaCN with 62% chlorine content 400 mg/kg b.w./day ^b (0.4% in diet)	Survival, Reduction in weight gain/loss of weight, Reduction in liver weight and Reduction in liver vitamin A content	400 mg/kg b.w./day ^b	-
12 week feeding study in female CF-E rats (n = 104 per group) ²⁴	Hexachloronaphthalene containing 61% chlorine 0, 2, 6.3 or 20 mg/kg b.w./day ^b (0, 0.002, 0.0063 or 0.02% in diet)	Relative liver weight	2 mg/kg b.w./day ^b (0.002% in diet)	-
Lifetime exposure study in hamsters using oral dosing (n = 5 per group) ²⁵	Haftax 1 with 52% chlorine content 10 to 20 mg/kg b.w./day	Reduction in feed consumption, Loss of weight, Dilatation of right heart, Congestion and oedema of lungs, Fatty degeneration of the liver	10 mg/kg b.w./day	-
Lifetime exposure study in hamsters using oral dosing (n = 5 per group) ²⁵	Haftax 2 with 65% chlorine content 10 to 20 mg/kg b.w./day	Reduction in feed consumption, Loss of weight, Dilatation of right heart, Congestion and oedema of lungs, Fatty degeneration of the liver	10 mg/kg b.w./day	-
Lifetime exposure study in sheep using oral dosing by gelatine capsules (n = 5 per group) ²⁶	Halowax 1014 - mixture of penta- and hexaCN 0, 1.1, 11.0 or 27.5 mg/kg b.w./day	Reduced survival, lesions of the GI tract, nodular appearance of liver and cirrhosis,	1.1 mg/kg b.w./day	-
40 day feeding study in 1 day old turkey poults (n = 8 male & 8 female per group) ²⁷	Halowax 1014 – mixture of penta- and hexaCN 0, 5, 10, 20, 50 or 100 ppm in diet	Decreased weight gain and enlarged liver	5 ppm in diet	-
40 day feeding study in 12 day old chicks (n = 10 male & 10 female per group) ²⁸	Halowax 1014 – mixture of penta- and hexaCN 0, 0.5, 2.5, 12.5, 62.5 or 312.5 mg/kg b.w./day ^b (0, 4, 20, 100, 500 or 2500 ppm diet)	Retarded weight gain	12.5 mg/kg b.w./day ^b (100 ppm in diet)	2.5 mg/kg b.w./day ^b (20 ppm in diet)
28 week feeding study in 1 day old chicks (n = up to 7 per group) ²⁸	Halowax 1014 – mixture of penta- and hexaCN 0, 0.5, 2.5 or 12.5 mg/kg b.w./day ^b (0, 4, 20 or 100 ppm diet)	Reduced egg hatchability	2.5 mg/kg b.w./day ^b (20 ppm in diet)	0.5 mg/kg b.w./day ^b (4 ppm in diet)
Developmental toxicity				
Oral gavage dosing on GD 14-16 to pregnant Wistar rats (n = 7 per group) ²⁹	1,2,3,4,6,7-hexaCN 0 or 1x10 ⁻³ mg/kg b.w./day	Early onset of spermatogenesis in male offspring	1x10 ⁻³ mg/kg b.w./day	-

^a Extrapolated using values given in the paper

^b Extrapolated using values from the JECFA guidelines³⁰

27. 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 alanine amino

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transferase (ALT) activity was detected while a slight significant increase was seen in serum sorbitol dehydrogenase (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 glutathione (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 rats²⁰.

28. Investigations on a mixture of penta- and hexachloronaphthalenes showed that rats tolerated daily doses of 5 or 10 mg for up to 5 weeks at which time dosing stopped. Those receiving 15 mg/day all died within 3 weeks of initiation of dosing²².

29. 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 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 control²⁴.

30. In a study to investigate the effects of PCNs on vitamin A, 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) significantly depressed 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, using default values from the JECFA guidelines³⁰), 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, using default values from the JECFA guidelines³⁰), 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 livers²¹.

31. One study compared the effects of a mixture of tetra-, penta- and hexachloronaphthalene congeners with 62% chlorine content in rats and hamsters.

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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 feed²³.

32. Six guinea pigs were given doses of synthetic pentachloronaphthalene from 2.5 to 20 mg/kg b.w./day for 6 days per week for their lifetime. 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 9²⁵.

33. Other rodent studies have shown increasing severity of effects elicited by PCNs with increasing chlorine content from tri- to hexa- congeners. 3 g of tri- congeners administered in the diet to 10 rats each day caused only slight changes in the liver whereas the same dose of a penta- and hexa- congener mixture caused weight loss and death within 1 month. Liver lesions included swelling, hypergranulation, hyaline inclusions and vacuolations with necrosis and fat accumulation³¹. Skin effects were elicited in rabbits initially exposed via the oral route followed by skin application when the animals were irradiated with a quartz lamp. This series of experiments also looked at the fat, phospholipid and cholesterol content of the liver of rats, mice and rabbits exposed to PCNs leading to the suggestion that egestion of fats from the liver via phospholipids is impaired as a result of PCN exposure³².

34. Two studies have been carried out in pigs where hexachloronaphthalene was administered in gelatin capsules. Reduction in vitamin A levels occurred 14 days after initiation of treatment but normal levels returned at time of necropsy. Reduction in weight gain and reduced survival occurred and necrosy showed degenerative liver changes. Other signs associated with bovine hyperkeratosis were not observed^{33,34}

35. 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 for their lifetime. 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

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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. 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 experiment²⁶.

36. A number of studies have been carried out in cattle using various PCNs. Lower chlorinated congeners showed few adverse effects. Penta- to octa- congeners caused hyperkeratosis and decreased survival. Pathological examination showed effects in liver, kidney and gastrointestinal tract in addition to wart-like proliferations in the mouth^{35,36,14,37}.

37. Effects of PCNs have also been studied in Broad Breasted Bronze turkey poults and New Hampshire Chickens. Decreased weight gain and liver enlargement were observed in both species and decreased egg hatchability was observed in the chickens as part of a study on effects on egg production^{27,28}.

Reproductive and Developmental studies

38. Two studies have been carried out assessing reproductive and developmental effects of PCNs. One study was carried out in pregnant Wistar rats which 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. No significant differences were observed except that advanced spermatids on PND 48 and caudal sperm count on PND 62 were increased in treated animals compared to controls. On PND 31 the percentage of postmeiotic tubules were increased and serum LH had reached a plateau and serum FSH had attained the maximum level both of which were earlier than in the control group. The evidence from this study indicated that hexachloronaphthalene accelerated the onset of sperm production in the testes²⁹. The other experiment studied the effects of a mixture of penta- and hexa- congeners on a yearling bull dosed for 7 weeks and observed for a year thereafter. Alongside signs of hyperkeratosis, no spermatozoa were present in the semen after 2 months and this continued for a further 6 months. Where sperm were present, various abnormalities were observed though these decreased with recovery. Microscopy of the left testis showed degeneration of the seminiferous epithelium and squamous metaplasia of the head of the epididymis. Prior to termination, the bull was successfully bred with 2 heifers, one by natural breeding and the other by artificial insemination³⁸.

Genotoxicity and carcinogenicity

39. 1,2,3,4-Tetrachloronaphthalene and 1-monochloronaphthalene were not mutagenic to *Salmonella typhimurium* strains in the Ames test with or without activation. No long-term or carcinogenicity studies have been identified¹.

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Epidemiology studies

40. 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 effects³¹.

41. A further three cases 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 vomiting³⁹.

42. 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 chloronaphthalene⁴⁰.

43. Case reports of seven people coming into contact with pentachloronaphthalenes suggest that papular rash 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 recovery⁴¹.

44. 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. Six 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 serum 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 GGT values were increased in 6 of the 16 workers; one person had increased bilirubin and another increased GPT. Two 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 changes in liver function could be a result of their exposure to PCNs in the workplace⁴².

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Relative Potency

45. 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). 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, although 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 intake⁴³. 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^{44,45}.

46. Several papers discuss the use of *in vitro* assays such as ethoxyresorufin O-deethylase (EROD) activity in fish and rat cell lines^{46,47,48,49,50}, DR-CALUX⁴⁶, H4IIE-luciferase^{51,49}, aryl hydrocarbon hydroxylase (AHH) and aryl hydrocarbon receptor (AhR) binding activity⁴⁷ to compare the activity of individual PCNs or mixtures of PCNs with 2,3,7,8-TCDD and in some cases to propose TEFs.

47. *In vivo* assays have been used to compare the activity of PCNs or mixtures by analysing liver enzyme activities, such as AHH^{52,53}, EROD^{52,54} or pentoxyresorufin O-deethylase (PROD)⁵⁴. In addition experiments have been carried out evaluating liver vitamin A, α -tocopherol and GSH content and liver GSH-S-transferase, GSH peroxidase, catalase, superoxide dismutase and hexose monophosphate shunt activity⁵⁴.

48. The available data have also been used to attempt *in silico* derivation of TEF values for all PCNs, for example, a recent paper used *in vitro* H4IIE EROD and luciferase assay data along with a large number of chemical descriptors to determine TEF values for all the chloronaphthalene congeners relative to 2,3,7,8-TCDD⁵⁵. A similar paper⁵⁶, 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) review². The authors 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, 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 AhR⁵⁷.

49. In the FSA investigation, the Hanberg *et al.* (1990)⁴⁷, Blankenship *et al.* (2000)⁵¹, Villeneuve *et al.* (2000)⁴⁹ and Behnisch *et al.* (2003)⁴⁶ EROD or luciferase assay values were used to determine the concentrations on the basis of relative

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potency to TCDD in the different foodstuffs analysed. Table 2 provides the values used for the investigation; where more than one value was available, the worst case relative potency was used.

Table 2: Relative potency of PCN congeners analysed in the FSA investigation of PCNs in food.

Congener	Name	Relative Potency compared to TCDD
PCN 52	1,2,3,5,7-PentaCN	<0.000025 ^a
PCN 53	1,2,3,5,8-PentaCN	<0.0000018 ^b
PCN 66/67	1,2,3,4,6,7-HexaCN/ 1,2,3,5,6,7-HexaCN	0.004 ^{*c} 0.003 ^d
PCN 68	1,2,3,5,6,8-HexaCN	0.0028 ^a
PCN 69	1,2,3,5,7,8-HexaCN	0.002 ^d
PCN71/72	1,2,4,5,6,8-HexaCN/ 1,2,4,5,7,8-HexaCN	0.000007 ^d 0.00009 ^{**b}
PCN 73	1,2,3,4,5,6,7-HeptaCN	0.0031 ^a
PCN 74	1,2,3,4,5,6,8-HeptaCN	0.0000041 ^b
PCN 75	OctaCN	0.00001 ^b

^a Blankenship *et al.* (2000)⁵¹

^b Behnisch *et al.* (2003)⁴⁶

^c Villeneuve *et al.* (2000)⁴⁹

^d Hanberg *et al.* (1990)⁴⁷

* This value was used for PCN 66/67

** This value was used for PCN 71/72

Estimates of dietary exposure

50. Dietary exposure 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 where concentrations in food were summed by weight of the congeners present. Children had the highest estimated dietary exposure of 1.65 ng/kg b.w./day and it was estimated that a 70 kg male would have a total dietary exposure of 45.78 ng/day or 0.65 ng/kg b.w./day⁵⁸. In a subsequent study, estimates of average dietary exposure were 56.63 ng/day and 38.00 ng/day for elite sportsmen and women, respectively, in Catalonia, Spain. For high consumers, the estimates were 72.31 ng/day for elite sportsmen and 47.08 ng/day for elite sportswomen⁵⁹.

51. Exposure to PCNs (tri- to octachloronaphthalene congeners summed on a weight basis) from consumption of 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 quantitation⁶⁰. The authors determined this to be equivalent to 0.00 and 0.01 pg WHO-TEQ/kg b.w./day, if the dioxin-like toxicity was estimated from the relative potency values reported by Blankenship *et al.*, 2000⁵¹ and Villeneuve *et al.*, 2000⁴⁹ (see para 46).

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52. In 2007, 42 composite samples of fish in Catalonia, Spain, were analysed for tetra- to octachloronaphthalenes. The total PCN intake from fish, where concentrations were summed by weight of the congeners present, for a standard male adult of 70kg was estimated to be 1.53 ng/day. The highest estimated exposure from fish was for boys: 0.04 ng/kg b.w./day⁶¹. Using these data and other 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 considered likely to be 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 old⁶². Children's intake from fish and seafood from the same area, surveyed in 2005, was estimated to be 0.04 ng/kg b.w./day for boys and 0.02 ng/kg b.w./day for girls⁶³.

Results of the FSA investigation

53. 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. 1,2,3,5,7-pentaCN (PCN 52) was always the most abundant congener in fish, a finding consistent with the survey in Spain⁶¹, but in other foods it was not as dominant. The higher concentrations in fish are consistent with the Falandysz (2003) review of PCNs in food². The main contributors in fish were 1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN (PCNs 66/67) making up 70% of the summed concentration. OctaCN (PCN 75) was detected only occasionally. In meat, offal and dairy products 1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN (PCNs 66/67) and 1,2,3,4,5,6,7-heptaCN (PCN 73) were detected more frequently.

54. The concentrations of all the congeners determined (1,2,3,5,7-pentaCN (PCN 52), 1,2,3,5,8-pentaCN (PCN 53), 1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN (PCNs 66/67), 1,2,3,5,6,8-hexaCN (PCN 68), 1,2,3,5,7,8-hexaCN (PCN 69), 1,2,4,5,6,8-hexaCN/1,2,4,5,7,8-hexaCN (PCNs 71/72), 1,2,3,4,5,6,7-heptaCN (PCN 73), 1,2,3,4,5,6,8-heptaCN (PCN 74) and octaCN (PCN 75)), summed on the basis of relative potency compared to TCDD, as identified in para 49, were 0.0001 - 0.02 ng/kg fresh weight. The concentrations of the PCNs summed on a weight basis were 0.15 - 37.3 ng/kg fresh weight.

55. The concentrations measured in the small number of samples taken may not be representative of the foodstuffs, and it is not possible to calculate a reliable estimate of UK dietary exposure. However, on an extreme worst case basis, if total food consumed by an adult (4.7 kg/day for the 97.5th percentile consumer⁶⁴) contained the PCNs in the range identified in the FSA investigation, on the basis of relative potency compared to TCDD, then the total dietary exposure would be 0.006 - 1.2 pg/kg b.w./day. The highest food consumption on the basis of body weight is by toddlers (1.5 - 4.5 year olds) (2.0 kg total food/day by the 97.5th percentile consumer⁶⁵). Using the same assumptions, their worst case exposure to PCNs on a basis of relative potency to TCDD would be 0.014 - 2.8 pg/kg b.w./day.

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Previous Risk Assessments

56. Two reviews have been carried out by national or international bodies, which examined the data on polychlorinated naphthalenes, however risk assessments as such have not been performed and instead risk management practices have been suggested by the IPCS in the 2001 Concise International Chemical Assessment Document¹ and in 1975 by the United States Environmental Protection Agency (US EPA)⁶⁶.

57. 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 PCBs⁶⁷.

COT Evaluation

58. The Committee noted that some PCN mixtures have shown clear evidence of dioxin-like activity, including induction of chloracne in exposed workers. Therefore a cumulative approach to risk assessment for dioxin-like compounds should also be applied to the PCNs.

59. According to van den Berg *et al.* (2006), the criteria for inclusion of a dioxin-like compound in the TEF concept are that it 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.

60. On this basis, it is currently not possible to establish TEFs for PCNs due to the lack of information on persistence. In addition there is a lack of repeat dose toxicity studies allowing direct comparison with TCDD. It is likely that some PCN congeners act via the AhR and the Committee considered that it is reasonable to assume that they are less persistent than the PCDDs and PCDFs. Therefore using the relative potencies identified from *in vitro* studies provides a conservative approach to cumulative risk assessment for the dioxin-like PCNs.

61. The maximum worst case dietary exposure of toddlers to PCNs, on the basis of dioxin-like activity, would be 0.7 - 140% of the Tolerable Daily Intake (TDI) of 2 pg WHO-TEQ/kg b.w.⁶⁸. High level dietary intake of dioxins and dioxin-like PCBs by 1.5 to 2.5 year olds in 2001 was estimated to be 4.2 pg WHO-TEQ/kg b.w./day using the 2005 WHO-TEFs⁶⁹. The maximum worst case dietary exposure to PCNs is 0.3 - 67% of this. In addition to using a worst case scenario, it is also expected that the relative potency values for PCNs compared to TCDD obtained from *in vitro* studies will

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overestimate the dioxin equivalent concentration as such assays cannot take into account the lower cumulative properties of PCNs compared to dioxins. Thus these extreme calculations do not indicate a health concern. An improved estimate of dietary exposure could be obtained using a Total Diet Study approach.

62. The evidence does not suggest that all the toxic effects of all PCNs occur through interaction with the AhR. The lowest LOAEL identified in the oral repeat dose studies was 1 µg/kg b.w./day causing accelerated onset of spermatogenesis. The margins of exposure between this LOAEL and the maximum worst case dietary exposure for adult where PCN concentrations in food are summed on a weight basis would be 435-108,000. This LOAEL is derived from an experiment using 1,2,3,4,6,7-hexachloronaphthalene and the evidence suggests that the hexa- congeners are likely to be more toxic than some of the other congeners, whereas summing on a weight basis assumes all the congeners are of equal potency. Thus this highly conservative comparison also does not indicate a health concern

COT Conclusions

63. We conclude that a cumulative approach to risk assessment for dioxin-like compounds should also be applied to the PCNs. The currently available data are inadequate to establish TEFs for PCNs, but in the absence of other data, relative potencies compared to TCDD from *in vitro* studies can be used as a highly conservative approach to cumulative risk assessment for some congeners. The identified relative potencies are given in Table 2

64. It is unlikely that all the toxic effects of PCNs occur through interactions at the AhR. Assessing the sum of PCN congeners using a margin of exposure approach compared to the LOAEL for effects of the most toxic PCN congener on sperm production also provides a conservative approach to cumulative risk assessment.

65. The concentrations measured in the small number of samples taken in the FSA investigation may not be representative of the foodstuffs, and it is not possible to calculate a reliable estimate of UK dietary exposure. However a maximum worst case dietary exposure assuming the total food consumed contains the concentrations of PCNs found in the investigation has been calculated.

66. Using these very conservative assumptions, the data from the FSA investigation suggest that maximum PCN exposure to toddlers would be to 0.7 - 140 % of the TDI of 2 pg WHO-TEQ/kg b.w for dioxins and dioxin-like compounds. While this suggests the TDI could be exceeded, it is likely that this approach over-estimates the potency of PCNs compared to dioxins. Taking into account the lower cumulative properties of PCNs compared to dioxins, these extreme calculations are likely to considerably overestimate dietary exposure to dioxin-like activity and do not indicate a health concern.

67. The margins of exposure between the lowest LOAEL and the maximum worst case dietary exposure were 435-108,000 for adults, which also do not indicate a health concern.

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68. Overall, although the data are insufficient for a robust risk assessment, the results of the FSA investigation do not suggest a concern for consumer health.

69. We would recommend that, if further work be carried out on these compounds, a Total Diet Study approach be used to obtain a more accurate estimation of human dietary exposure. However, there would still be considerable uncertainty in any risk assessment as the toxicological database is insufficient for deriving health-based guidance values.

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Annex A

Search Strategy

General Polychlorinated Naphthalene Search September 2006

Databases interrogated –

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

Search Terms – (polychlorinated-naphthalene*) 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
- Were exposure assessments or contained mainly survey data
- Concentrated on sampling, measurement, analysis and screening methods
- Were not concerned with toxicity
- Focused on eco-toxicity

Additional Polychlorinated Naphthalene Search October 2008

Databases interrogated –

- PubMed

Search Terms – (polychlorinated naphthalene*) 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
- Focussed on temporal trends
- Were exposure assessments or contained mainly survey data Focussed on environmental contamination and/or bioaccumulation
- Concentrated on sampling, measurement, analysis and screening methods
- Did not consider Polychlorinated Naphthalene's (PCN's) in any detail

Further Polychlorinated Naphthalene Search October 2008

Databases interrogated –

- PubMed

Search Terms – (Chloro* Naphthalene OR Polychloro* Naphthalene OR Chlorinated Naphthalene) 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
- Focussed on temporal trends
- Were exposure assessments or contained mainly survey data

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- Focussed on environmental contamination and/or bioaccumulation
- Concentrated on sampling, measurement, analysis and screening methods
- Did not consider Polychlorinated Naphthalene's (PCN's) in any detail
- Were not concerned with toxicity

Fourth Polychlorinated Naphthalene 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
- Focussed on temporal trends
- Were exposure assessments or contained mainly survey data
- Focussed on environmental contamination and/or bioaccumulation
- Concentrated on sampling, measurement, analysis and screening methods
- Did not consider Polychlorinated Naphthalene's (PCN's) in any detail
- Were not concerned with toxicity
- Were duplicated papers or ones already held

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.