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

Second 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. The first draft of this statement on polychlorinated naphthalenes in food based on the discussions and conclusions at the February 2009 meeting was discussed in April 2009.

2. Following discussion and comments at the April 2009 meeting and afterwards, a second draft statement has been prepared and is attached at Annex A.

Questions on which the views of the Committee are sought

3. Members are invited to agree the content of the draft statement.

Secretariat
April 2009

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

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Second Draft Statement on Polychlorinated Naphthalenes in Food

Introduction

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

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 1[1].

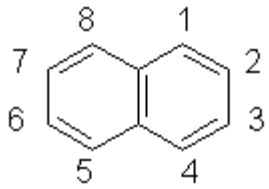

Figure 1: Naphthalene structure and the positions where chlorine atoms can be substituted.

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 products during waste incineration and may also be released when products containing PCNs are disposed of to landfill[1].

4. In the 1930s and 1940s, PCNs were found to be associated with chloracne and, in some instances, liver disease in exposed workers. Later they were shown to

cause bovine hyperkeratosis, a disorder of cattle with significant economic impact in several countries. This disease was produced through interference with the biotransformation of carotene to vitamin A[1].

5. Resistance of PCNs to biotic degradation, and hence persistence in the environment, increases with increasing chlorine content and depends on the number of adjacent carbon atoms unsubstituted with chlorine[2]. 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. Papers relevant to this review were identified using the search strategy outlined in Annex A.

Toxicokinetics

Absorption

6. From studies on the toxicokinetics 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 appear to be very poorly absorbed[1]. 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. Blood levels gradually declined over the first 8 hours. By 48 hours, radioactivity levels in blood were approximately 20% of the peak 1 hour concentration[3].

Distribution

7. Groups of 3 male JCL:ICR mice were given a single intraperitoneal (i.p.) dose of 1mg 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 administered 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 for octachloronaphthalene in adipose tissue, levels of which increased gradually up to day 7. The biological half-life of all three compounds was longest in 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 both the positions and the numbers of chlorine atoms influence the biological half-life[4].

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 detected only in the skin and adipose tissue and corresponded to 0.05% of the total dose[3].

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- congener[5].

10. Technical mixtures of PCNs have been used to study retention and redistribution. 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 might be explained by lower absorption. A hexa- congener was dominant in the intraperitoneal fat from days 10 to 120 and at day 120 this was the only congener detected at a concentration 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 identified in liver and/or adipose tissue (not specified which)[6].

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 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 the compounds 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 kidney[7].

12. Samples of human adipose tissue and liver from Swedish subjects (5 male and 2 females, aged 47 – 80 years old) who had died suddenly 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 found[8].

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 compounds[9].

14. Three rabbits were given a single 300 mg/kg dose of 2,6-dichloronaphthalene (route not stated) and four Sprague Dawley rats were given a single 1 g/kg dose, following which urine was collected for a week. Pooled urine samples from each species contained unchanged 2,6-dichloronaphthalene, a monochloronaphthol derivative and a dichloronaphthol derivative[10].

15. Groups of 3 male albino rabbits were given a single 1 g 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, but the total 4 day excretion did not exceed 20% of the 1 g dose. The authors suggested this indicated that the penta-, hepta- and octa- congeners do not undergo metabolism to these conjugates[11].

16. Pigs were administered 30 mg chloronaphthalenes/kg b.w. by retrocarotid injection in corn oil. After 5 hours animals were sacrificed and urine was 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-naphthol[12].

17. 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 urine collected over this period and bile were analysed. 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 administration of 1,2,3,4,5,6-hexachloronaphthalene[13].

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 bile[7].

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

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 dihydrodiol[3].

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 congeners[5].

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 mother (reduced milk flow was noted from day 7 and ceased at day 29). Autopsy of the calf on day 50 after the start of treatment showed moderate toxicity suggesting that the octachloronapthalene or its metabolites may be excreted in the milk[14].

22. The half-lives of hexachloronaphthalene in rat liver and adipose tissue have been determined as 36 and 41 days respectively[15]. Human blood samples from 3 individuals taken at a number of time points after the Yu-cheng rice oil incident in 1979, when heat exchange media leaked into food grade oil resulting in contamination with a range of polychlorinated substances including PCNs. The sampes were analysed for tetra-, penta- and hexa- PCN content; only the 1,2,3,4,6,7- and/or 1,2,3,5,6,7-hexa- congeners (which co-elute by gas-chromatography) were detected and the half-life was estimated to be 1.5-2.4 years[16]. An analysis of human milk samples collected between 1972 and 1992 from healthy native Swedish mothers living in the Stockholm region, without a specific source of PCN exposure, decreased from 3.08 to 0.48 ng/g lipid for the sum of three tetra-congeners (including 1,2,5,6-tetraCN), 1,2,3,5,7-pentaCN, 1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN, 1,2,4,5,6,8-hexaCN/1,2,4,5,7,8-hexaCN and 1,2,3,4,5,6-hexa CN[17]. The authors calculated that the concentration halved over 8 years, which is likely to reflect both elimination and reducing environmental exposure [18]. Analysis of human adipose tissue samples, collected between 2003 and 2005 from 43 individuals (29 females and 14 males) living in New York with no specific source of PCN exposure, showed concentrations, summed on a weight basis, of tri- to octa- CNs of 21-910 pg/g lipid weight for females (mean 270 ± 220 pg/g lipid weight) and 61-2500 pg/g lipid weight for males (mean 590 ± 620 pg/g lipid weight)[19].

Toxicity

23. The database on PCNs is heterogeneous as studies have been carried out on different congeners or commercial mixtures and in different species. The responses

elicited have varied according to 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. Investigations in sheep and pigs then 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 were observed in such studies which are in line with the effects seen in cattle (bovine hyperkeratosis) and humans (chloracne)[1]. 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[2].

Acute toxicity

25. Three acute toxicity studies have been carried out. A 2008 study in rats used a tetra-hepta congener mixture. At all doses (250-1000 mg/kg b.w.), there was a reduction in bodyweight, and 72 hours after dosing with 500 and 1000 mg/kg b.w., an increase in relative liver mass. Increasing liver cytochrome P450 (CYP) content and CYP1A activity were observed. Increasing liver malondialdehyde levels and a slight but significant elevation in serum sorbitol dehydrogenase led the authors to suggest that the mechanism of PCNs' toxic action is probably associated with oxidative stress induction due to changes in malondialdehyde[20]. 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 toxicity[11]. Single doses of penta- (22 mg/kg b.w.) or hexa- (11 mg/kg b.w.) congeners administered orally to calves in gelatine capsules caused hyperkeratosis, and at autopsy, gross and microscopic lesions of the liver and kidney were seen[21].

Repeat dose toxicity

26. Table 1 provides a summary of the most sensitive effects observed in repeat dose and developmental studies using oral administration. For repeat dose exposure, studies in laboratory species are included if more than one dose group was tested. For the farm species, repeat dose studies were summarised if the doses were at or below the doses given in laboratory animals, more than one animal was tested per dose, and the dose given was consistent throughout the study.

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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)[22]	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)[23]	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)[24]	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)
8 week feeding study in Sprague Dawley rats (n = 3 to 4 per group)[25]	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)[25]	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)[26]	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)[27]	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)[27]	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)[28]	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)[29]	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)[30]	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)[30]	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)

Type of study	Substance and dose	Most sensitive endpoint	LOAEL	NOAEL
Developmental toxicity				
Oral gavage dosing on GD 14-16 to pregnant Wistar rats (n = 7 per group)[31]	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 guidelines[32]

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 food and water consumption with a related decrease in body weight by 15-30% of the initial body mass, such 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 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 indicated induction of lipid peroxidation and oxidative stress in the livers of these rats[22].

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 dosing[24].

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 sleeping time following administration of hexobarbital (a marker for induction of CYP enzymes) or sensitivity to pentamethylenetetrazole-induced convulsions. The day after these tests, animals were autopsied. Animals receiving 0.02% in the diet (20mg/kg b.w./day) showed decreased food consumption and depression of body weight and weight gain. Moreover, 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. No change in sensitivity to pentamethylenetetrazole occurred in any group, indicating an absence of marked depressant effects on the central nervous system, whilst hexobarbital sleep time was increased at 4 and 8 weeks in the 20 mg/kg b.w./day group[26].

30. In a study to investigate the effects of PCNs on vitamin A stores, 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 guidelines[32]), 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 guidelines[32]), 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[23].

31. 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. Two animals in the high dose group died before the end of the experiment. Average weight gain and liver weight were 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[25].

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 (route assumed to be oral). 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[27].

33. Other rodent studies have shown increasing severity of effects elicited by PCNs with increasing chlorine content from tri- to hexa- congeners. Administration of 3 g of tri- congeners in the diet to 10 rats (i.e. an average of 0.3g per rat) 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 accumulation[33]. Dermal application of PCNs resulted in skin photoirritation in rabbits pretreated via the oral route (0.4 g per rabbit for up to 3 months), but not without the oral pre-treatment. This series of experiments also found impaired fat metabolism in the liver of rats, mice and rabbits exposed to unspecified doses of PCNs in the diet[34].

34. Two studies have been carried out in pigs where hexachloronaphthalene was administered orally in gelatin capsules. Reduction in serum vitamin A levels occurred 14 days after initiation of treatment but normal levels had 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[35,36]

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 per os 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 killed. 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 deficiency in this vitamin. 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 experiment[28].

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[37,38,14,39].

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[29,30]

Reproductive and Developmental studies

38. Two studies have assessed reproductive and developmental effects of PCNs. 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 and effects on the reproductive systems of the male offspring were examined. Anogenital distance and

time of eye-opening were unaffected, but statistically significant increases in serum luteinising hormone (LH), serum follicle-stimulating hormone (FSH) and the percentage of postmeiotic tubules on postnatal day (PND) 31, seminal vesicle weight and advanced spermatids on PND 48, and caudal sperm count on PND 62 indicated that hexachloronaphthalene accelerated the onset of spermatogenesis[31]. A mixture of penta- and hexa- congeners was given in a daily oral to a yearling bull for 7 weeks (50-200 mg per day, cumulative dose of 1.8 g) followed by observation 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 insemination[40].

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 identified[1].

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 effects[33].

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 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 had jaundice for 2 months prior to admission to hospital. Two weeks before admission this had become worse and he had developed abdominal pain, malaise, nausea and vomiting[41].

42. Twelve workers in a chromium plating factory with exposure to dust or 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[42].

43. Case reports of seven people coming into contact with penta- congeners suggest that papular rash or depigmentation are the most common early symptoms of toxicity. Liver damage is a possible consequence of continued exposure, but removal from contact at the time of gastrointestinal symptoms led to rapid recovery[43].

44. A study of 16 workers exposed to wax fumes, of which 90% were 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 had suffered a stroke, and another had had renal and bladder cancer), and 2 others had their serum gamma-glutamyl transferase (GGT) levels measured (one of whom had laryngeal cancer). Three 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 glutamic pyruvic transaminase (GPT). Two workers had fatty livers. Other parameters such as electrocardiogram, X-ray of the chest and laboratory measurements were not indicative of pathology 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 workplace[44].

Aryl hydrocarbon receptor-mediated Toxicity and Relative Potency

45. *In vivo* and *in vitro* studies have shown that PCNs induce dioxin-like effects and aryl hydrocarbon receptor (AhR)-mediated activities, in a manner analogous to PCDDs and PCDFs, although the PCNs are less potent. These data, together with their structural similarity to dioxins, suggests that Toxic Equivalency Factors (TEFs) could be derived for the different PCN congeners to enable determination of 2,3,7,8-TCDD Equivalents (TEQ), which is a measure of AhR-mediated toxicity. 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 determination of TEF values. It was noted that possible contamination of PCNs with small amounts of other dioxin-like compounds had 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[45]. 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[46,47].

46. Several papers discuss the use of *in vitro* assays such as 7-ethoxyresorufin O-deethylase (EROD) activity in fish and rat cell lines[48,49,50,51,52], DR-CALUX[48], H4IIE-luciferase[53,51], aryl hydrocarbon hydroxylase (AHH) and AhR binding activity[49] 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. Measurement of enzyme activities, such as AHH[54,55], EROD[54,56] or 7-pentoxyresorufin O-depentylase (PROD), GSH-S-transferase, GSH peroxidise, catalase and superoxide dismutase, and hexose monophosphate shunt activity,

vitamin A, α-tocopherol and GSH content [56] in livers of rats dosed intraperitoneally with PCNs have been used to compare the activity of PCN congeners or their mixtures..

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-TCDD[57]. A similar paper[58], 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[2]. 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 AhR[59].

49. In the FSA investigation, the Hanberg *et al.* (1990)[49], Blankenship *et al.* (2000)[53], Villeneuve *et al.* (2000)[51] and Behnisch *et al.* (2003)[48] EROD or luciferase assay values were used to determine the concentrations on the basis of relative 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]
PCN 71/72	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)[53]
[b] Behnisch *et al.*(2003)[48]
[c] Villeneuve *et al.* (2000)[51]
[d] Hanberg *et al.* (1990)[49]
* 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./day[60]. 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 sportswomen[61].

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 quantitation[62]. 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.*, 2000[53] and Villeneuve *et al.*, 2000[51] (see para 46).

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./day[63]. 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 old[64]. 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 girls[65].

Results of the FSA investigation

53. Forty five samples of various food types were analysed to ascertain the level at which PCNs are present in food on sale in the UK. Edible portions of each sample were homogenised, extracted and purified. High resolution gas chromatography coupled with high resolution mass spectrometry was used to determine levels of 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) in the samples[66].

54. 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 Spain[63], 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[2]. 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.

55. The concentrations of all the congeners, 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.

56. 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 all solid food consumed by an adult (1.5 kg/day for the 97.5th percentile consumer[67]) 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 equivalent to 0.002-0.39 pg/kg b.w./day of TCDD. The highest food consumption on the basis of body weight is by 4-6 year olds (1.0 kg total food/day by the 97.5th percentile consumer[68]). Using the same assumptions, their worst case exposure to PCNs on a basis of relative potency to TCDD would be equivalent to 0.005-0.98 pg/kg b.w./day of TCDD.

Previous Risk Assessments

57. 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 Document[1] and in 1975 by the United States Environmental Protection Agency (US EPA)[69].

58. 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 according to routes of exposure. It was noted that many of the data were produced to study the nature of the response in a variety of animal species, rather than to determine 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[70].

COT Evaluation

59. 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 was considered for application to the PCNs in order to be protective of public health.

60. 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.

61. On this basis, it is currently not possible to establish TEFs for PCNs due to the lack of information on persistence and the lack of repeat dose toxicity studies that would be needed for accurate and direct comparison with TCDD. It is likely that some PCN congeners can activate the AhR and therefore could have a cumulative effect (dose addition) with PCDDs and PCDFs. 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 an extremely conservative approach to cumulative risk assessment for the dioxin-like toxicity of PCNs.

62. The maximum worst case dietary exposure of 4-6 year olds to PCNs, on the basis of dioxin-like activity, would be 0.25 - 49% of the Tolerable Daily Intake (TDI) of 2 pg WHO-TEQ/kg b.w[71]. High level dietary intake of dioxins and dioxin-like PCBs by 4-6 year olds in 2001 was estimated to be 2.8 pg WHO-TEQ/kg b.w./day using the 2005 WHO-TEFs[72]. The maximum worst case dietary exposure to PCNs is 0.2 - 35% 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 overestimate the dioxin equivalent concentration as such assays cannot take into account the probable lower accumulative properties of PCNs compared to dioxins. Therefore, this estimation indicates dietary exposure to dioxin-like activity from PCNs is minor compared with that from other dioxin-like compounds and does not indicate a health concern for dioxin-like toxicity from PCNs at the levels found in the FSA investigation. An improved estimate of dietary exposure could be obtained using a Total Diet Study approach.

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63. The evidence suggests that some of the toxic effects of PCNs occur through mechanism(s) not involving interaction with the AhR. The Committee did not consider that any of the studies carried out on PCNs assessed the range of endpoints required to carry out a full hazard characterisation. The Committee also noted that the position of chlorine substitution and the degree of chlorination are likely to have a substantial impact on the effects resulting from the different congeners. However, the available data did not indicate specific toxicological concerns.

COT Conclusions

64. We conclude that because some PCN mixtures exhibit dioxin-like activity, protection of public health requires a cumulative approach to risk assessment. 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 indicate if dioxin-like activity of PCNs in food present a risk to the consumer.

65. It is unlikely that all the toxic effects of PCNs occur through interactions at the AhR, however we do not consider any of the studies on PCNs are sufficient to carry out a full hazard characterisation

66. 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 that all solid food consumed contains the concentrations of PCNs found in the investigation has been calculated.

67. Using these very conservative assumptions, the data from the FSA investigation suggest that maximum PCN exposure to 4-6 year olds would be to 0.25 - 49 % of the TDI of 2 pg WHO-TEQ/kg b.w for dioxins and dioxin-like compounds, and could add 0.2 - 35% to the total dietary exposure for this age group.

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68. Overall, although the data are insufficient for a robust risk assessment, the results of the FSA investigation do not raise specific toxicological concerns.

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. However further research to improve the toxicological database is not a priority as these compounds are no longer deliberately produced and only low levels have been detected in food.

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References

1 IPCS. (2001). Chlorinated Naphthalenes. *CICAD* 34.
2 Falandysz, J. (2003). Chloronaphthalenes as food-chain contaminants: a review. *Food Additives and Contaminants* 20[11], 995-1014.
3 Chu, I., Secours, V., Villeneuve, D.C., Viau, A. (1977). Metabolism and tissue distribution of (1,4,5,8-14C)--1,2-dichloronaphthalene in rats. *Bull Environ Contam Toxicol* 18: 177-183.
4 Oishi, H. and Oishi, S. (1983). Tissue distribution and elimination of chlorinated naphthalenes in mice. *Toxicol Lett* 15: 119-122.
5 Kilanowicz, A., Galoch, A., Sapota, A. (2004). Tissue distribution and elimination of selected chlorinated naphthalenes. *Int J Occup Med Environ Health* 17: 355-360.
6 Asplund, L., Jannson, B., Sundstrom, G. (1986). Characterisation of a strongly bioaccumulating hexachloronaphthalene. *Chemosphere* 15, 619-628.
7 Ruzo, L.O., Jones, D., Platonow, N. (1976). Uptake and distribution of chloronaphthalenes and their metabolites in pigs. *Bull Environ Contam Toxicol* 16: 233-239.
8 Weistrand, C. and Noren, K. (1998). Polychlorinated naphthalenes and other organochlorine contaminants in human adipose and liver tissue. *J Toxicol Environ Health A* 53: 293-311.
9 Chu, I., Villeneuve, D.C., Secours, V., Viau, A. (1977). Metabolism of chloronaphthalenes. *J Agric Food Chem* 25: 881-883.
10 Chu, I., Secours, V., Viau, A. (1976). Metabolites of Chloronaphthalene. *Chemosphere* 6, 439-444.
11 CORNISH, H.H. and BLOCK, W.D. (1958). Metabolism of chlorinated naphthalenes. *J Biol Chem* 231: 583-588.
12 Ruzo, L. O., Safe, S., Hutzinger, O., Platonow, N., Jones, D. (1975). Hydroxylated metabolites of chloronaphthalenes (Halowax 1031) in pig urine. *Chemosphere* 3, 121-123.
13 Ruzo, L., Jones, D., Safe, S., Hutzinger, O. (1976). Metabolism of chlorinated naphthalenes. *J Agric Food Chem* 24: 581-583.
14 Sikes, D., WISE, J.C., BRIDGES, M.E. (1952). The experimental production of X disease (hyperkeratosis) in cattle with chlorinated naphthalenes and petroleum products. *J Am Vet Med Assoc* 121: 337-344.
15 Asplund, L., Jakobsson, E., Haglund, P., Bergman, A. (1994). 1,2,3,5,6,7-Hexachloronaphthalene and 1,2,3,4,6,7-Hexachloronaphthalene - Selective Retention in Rat-Liver and Appearance in Wildlife. *Chemosphere* 28: 2075-2086.
16 Ryan, J. J. and Masuda, Y. (1994). Polychlorinated naphthalenes (PCNs) in the rice oil poisonings. *Organohalogen Compounds* 21, 251-254.
17 Lunden, A. and Noren, K. (1998). Polychlorinated naphthalenes and other organochlorine contaminants in Swedish human milk, 1972-1992. *Arch Environ Contam Toxicol* 34: 414-423.
18 Noren, K. and Meironyte, D. (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere* 40: 1111-1123.
19 Kunisue, T., Johnson-Restrepo, B., Hilker, D.R., Aldous, K.M., Kannan, K. (2009). Polychlorinated naphthalenes in human adipose tissue from New York, USA. *Environ Pollut* 157: 910-915.

20 Galoch, A., Sapota, A., Skrzypinska-Gawrysiak, M., Kilanowicz, A. (2006). Acute toxicity of polychlorinated naphthalenes and their effect on cytochrome P450. *Human & Experimental Toxicology* 25, 85-92.
21 BELL, W.B.(1954). The production of hyperkeratosis (X disease) by a single administration of chlorinated naphthalenes. *J Am Vet Med Assoc* 124: 289-290.
22 Kilanowicz, A., Skrzypinska-Gawrysiak, M., Sapota, A., Galoch, A., Darago, A. (2008). Subacute toxicity of polychlorinated naphthalenes and their effect on cytochrome P-450. *Ecotoxicol Environ Saf*
23 DEADRICK, R.E., BIERI, J.G., CARDENAS, R.R., Jr. (1955). Effects of octachloronaphthalene on vitamin A metabolism in the rat. *J Nutr* 57: 287-295.
24 Cleary, R. V., Maier, J., Hitchings, G. H. (1938). The metabolism of Chlorinated Naphthalenes. *J Biol Chem* , 403-409.
25 SCHOETTLE, C.E., REBER, E.F., MORRILL, C.C., Link, R.P. (1955). Experimental production of hyperkeratosis in rats and hamsters. *Am J Vet Res* 16: 183-188.
26 Weil, C.S. and GOLDBERG, M.E. (1962). Toxicological and pharmacological criteria of repeated doses of a hepatotoxic agent. *Acta Pharmacol Toxicol (Copenh)* 19: 129-138.
27 Bentz, H. and HERDMANN, I. (1955). Suitability of the guinea pig as a test animal for the identification of cases of poisoning by chlorinated naphthalenes. *Institute of Veterinary Pharmacology and Toxicology* .
28 BROCK, W.E., JONES, E.W., MACVICAR, R., POPE, L.S. (1957). Chlorinated naphthalene intoxication in sheep. *Am J Vet Res* 18: 625-630.
29 Pudelkiewicz WJ, e. a. (1958). Some physiological responses of broad breasted bronze poults to chlorinated naphthalenes. *Poultry Sci* 37, 185-187.
30 Pudelkiewicz WJ, e. a. (1959). Some physiological responses of New Hampshire chickens to a mixture of penta- and hexachloronaphthalenes. *Poultry Sci* 38, 424-430.
31 Omura, M., Masuda, Y., Hirata, M., Tanaka, A., Makita, Y., Ogata, R., Inoue, N. (2000). Onset of spermatogenesis is accelerated by gestational administration of 1,2,3,4,6,7-hexachlorinated naphthalene in male rat offspring. *Environ Health Perspect* 108: 539-544.
32 JECFA. (2001). Guidelines for the Preparation of Working Papers for the Joint FAO/WHO Expert Committee on Food Additives.
33 Drinker, C. K., Warren, M. F., Bennett, G. A. (1937). The problem of possible systemic effects from certain chlorinated hydrocarbons. *Journal of Industrial Hygiene and Toxicology* 19, 283-311.
34 Shakahnovskava FB. (1953). Toxicology of chlorinated naphthalenes. *Farmakol Toksikol* 16, 43-47.
35 HUBER, W.G. and Link, R.P. (1962). Toxic effects of hexachloronaphthalene on swine. *Toxicol Appl Pharmacol* 4: 257-262.
36 Link, R.P., SMITH, J.C., NEWTON, D.I. (1958). Toxic effect of chlorinated naphthalenes in pigs. *J Am Vet Med Assoc* 133: 83-85.
37 BELL, W. B. (1952). Further Studies on the Production of Bovine Hyperkeratosis by the Administration of a Lubricant. *The Virginia Journal of Science* 3, 169-177.

38 BELL, W. B. (1953). The relative toxicity of the chlorinated naphthalenes in experimentally produced bovine hyperkeratosis (X-disease). *Veterinary Medicine* 48, 135-146.
39 Sikes, D. and BRIDGES, M.E. (1952). Experimental production of hyperkeratosis ("X disease") of cattle with a chlorinated naphthalene. *Science* 116: 506-507.
40 VLAHOS, K., McENTEE, K., OLAFSON, P., HANSEL, W. (1955). Destruction and restoration of spermatogenesis in a bull experimentally poisoned with highly chlorinated naphthalene. *Cornell Vet* 45: 198-209.
41 Greenburg L, e. a. (1939). The systemic effects resulting from exposure to certain chlorinated hydrocarbons. *J Ind Hyg Toxicol* 21, 29-38.
42 Collier, E. (1943). Poisoning by Chlorinated Naphthalene. *The Lancet* 244, 72-74.
43 Cotter, L. H. (1944). Pentachlorinated Naphthalenes in Industry. *JAMA* 125, 273-274.
44 Popp, W., Hamm, S., Vahrenholz, C., Balfanz, E., Kraus, R., Theisen, J., Schell, C., Norpoth, K. (1994). Liver dysfunction in workers exposed to polychlorinated naphthalenes. *C R Acad Bulg Sci* 47, 117-120.
45 van den, B.M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E. (2006). The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci* 93: 223-241.
46 Falandysz, J. (1998). Polychlorinated naphthalenes: an environmental update. *Environmental Pollution* 101, 77-90.
47 McKinney, J. and McConnell, E. (1982). Structural specificity and the dioxin receptor. 367-381. Pergammon Press.
48 Behnisch, P.A., Hosoe, K., Sakai, S. (2003). Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environ Int* 29: 861-877.
49 Hanberg, A., Waern, F., Asplund, L., Haglund, E., Safe, S. (1990). Swedish Dioxin Survey - Determination of 2,3,7,8-Tcdd Toxic Equivalent Factors for Some Polychlorinated-Biphenyls and Naphthalenes Using Biological Tests. *Chemosphere* 20: 1161-1164.
50 Hanberg, A., Stahlberg, M., Georgellis, A., de Wit, C., Ahlborg, U.G. (1991). Swedish dioxin survey: evaluation of the H-4-II E bioassay for screening environmental samples for dioxin-like enzyme induction. *Pharmacol Toxicol* 69: 442-449.
51 Villeneuve, D.L., Kannan, K., Khim, J.S., Falandysz, J., Nikiforov, V.A., Blankenship, A.L., Giesy, J.P. (2000). Relative potencies of individual polychlorinated naphthalenes to induce dioxin-like responses in fish and mammalian in vitro bioassays. *Arch Environ Contam Toxicol* 39: 273-281.
52 Villeneuve, D. L., Khim, J. S., Kannan, K., Giesy, J. P. (2001). In vitro response of fish and mammalian cells to complex mixtures of polychlorinated naphthalenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. *Aquatic Toxicology* 54, 125-141.
53 Blankenship, A. L., Kannan, K., Villalobos, S. A., Villeneuve, D. L., Falandysz, J., Imagawa, T., Jakobsson, E, Giesy, J. P. (2000). Relative Potencies of Individual Polychlorinated Naphthalenes and Halowax Mixtures to Induce

Ah Receptor-Mediated Responses. *Environmental Science and Technology* 34[15], 3153-3158.

54 Campbell, M. A., Bandiera, S., Robertson, L., Parkinson, A., Safe, S. (1983). Hepta-, hexa-, tetra- and dichloronaphthalene congeners as inducers of hepatic microsomal drug-metabolizing enzymes. *Toxicology* 26, 193-205.

55 Cockerline, R., Shilling, M., Safe, S. (22-8-1980). Polychlorinated naphthalenes as hepatic microsomal enzyme inducers in the immature male rat. *Gen.Pharmac.* 12, 83-87.

56 Mãntylã, E. and Ahotupa, M. (1993). Polychlorinated biphenyls and naphthalenes: Long-lasting induction of oxidative stress in the rat. *Chemosphere* 27, 383-390.

57 Puzyn, T., Falandysz, J., Jones, P.D., Giesy, J.P. (2007). Quantitative structure-activity relationships for the prediction of relative in vitro potencies (REPs) for chloronaphthalenes. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 42: 573-590.

58 Falandysz, J. and Puzyn, T. (2004). Computational prediction of 7-ethoxyresorufin-O-diethylase (EROD) and luciferase (luc) inducing potency for 75 congeners of chloronaphthalene. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 39: 1505-1523.

59 Olivero-Verbel, J., Vivas-Reyes, R., Pacheco-Londono, L., Johnson-Restrepo, B., Kannan, K. (2004). Discriminant analysis for activation of the aryl hydrocarbon receptor by polychlorinated naphthalenes. *Journal of Molecular Structure-Theochem* 678: 157-161.

60 Domingo, J. L., Falcò, G., Llobet, J. M., Casas, C., Teixidó, A., Müller, L. (2003). Polychlorinated Naphthalenes in Foods: Estimated Dietary Intake by the Population of Catalonia, Spain. *Environmental Science and Technology* 37, 2332-2335.

61 Falco, G., Bocio, A., Llobet, J.M., Domingo, J.L. (2005). Health risks of dietary intake of environmental pollutants by elite sportsmen and sportswomen. *Food Chem Toxicol* 43: 1713-1721.

62 Jiang, Q., Hanari, N., Miyake, Y., Okazawa, T., Lau, R.K., Chen, K., Wyrzykowska, B., So, M.K., Yamashita, N., Lam, P.K. (2007). Health risk assessment for polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans, and polychlorinated naphthalenes in seafood from Guangzhou and Zhoushan, China. *Environ Pollut* 148: 31-39.

63 Llobet, J.M., Falco, G., Bocio, A., Domingo, J.L. (2007). Human exposure to polychlorinated naphthalenes through the consumption of edible marine species. *Chemosphere* 66: 1107-1113.

64 Marti-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L. (2008). Human exposure to polychlorinated naphthalenes and polychlorinated diphenyl ethers from foods in Catalonia, Spain: temporal trend. *Environ Sci Technol* 42: 4195-4201.

65 Marti-Cid, R., Bocio, A., Llobet, J.M., Domingo, J.L. (2007). Intake of chemical contaminants through fish and seafood consumption by children of Catalonia, Spain: health risks. *Food Chem Toxicol* 45: 1968-1974.

66 Food Standards Agency. (2009). Polychlorinated naphthalenes and chlorinated paraffins in food. *FSIS* .

67 Henderson, L., Gregory, J., Swan, G. (2002). National Diet and Nutrition Survey: Adults aged 19 - 64 Years. Volume 1: types and quantities of foods consumed. TSO.

68 Gregory, J., Lowe, S., Bates, C. J., Prentice, A., Jackson, L. V., Smithers, G., Wenlock, R., Farron, M. (2000). National Diet and Nutrition Survey: Young people aged 4 to 18 years, Volume 1: Report of the diet and nutrition survey.
69 USEPA. (1975). Environmental hazard assessment report : Chlorinated naphthalenes. United States Environmental Protection Agency.
70 USEPA. (1980). Ambient water quality criteria for chlorinated naphthalenes. i-C45. United States Environmental Protection Agency.
71 COT. (2001). COT statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls. *COT Statement* .
72 COT. (2006). 2005 WHO Toxic Equivalency Factors For Dioxins and Dioxin-like Compounds. *COT Statement* .

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

Annex A

Search Strategy

General Polychlorinated Naphthalene Search

Databases interrogated –

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

Search Terms – (polychlorinated-napthalene*) 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

General Polychlorinated Naphthalene Update Search

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 January 2000 to April 2009
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

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

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- 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) – to 15th April 2009

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.

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