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

Second draft statement of potential risks of α-, β- and γ-hexachlorocyclohexanes in the infant diet

Introduction

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that bears on the Government's dietary recommendations for infants and young children. The review will identify new evidence that has emerged since the Government's current recommendations were formulated, and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years, but will be considered in two stages, focussing first on infants aged 0 – 12 months, and then on advice for children aged 1 to 5 years. SACN is examining the nutritional basis of the advice, and has asked that evidence on possible adverse effects of diet should be considered by other advisory committees with relevant expertise. SACN asked COT to review the risks of toxicity from chemicals in the infant diet. The COT considered that persistent organic pollutants included in the Stockholm convention since 2009 including α-, β- and γ-hexachlorocyclohexanes (HCHs), should be included in this review.

2. There are currently no Government recommendations on complementary and young child feeding that relate to HCHs.

3. Lindane (γ-HCH) has been evaluated by the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) (WHO-IPCS, 1991) and by the Joint Food and Agriculture Organization (FAO)/WHO Meeting on Pesticide Residues (JMPR) (FAO/WHO, 2002). In addition, α- and β-HCH have been reviewed by IPCS (WHO-IPCS, 1992). The European Food Safety Authority (EFSA) has published an opinion on γ-HCH and other HCHs as contaminants in animal feed (EFSA, 2005). The US Environmental Protection Agency (USEPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) published reviews of HCH isomers toxicity (USEPA, 2001; ATSDR, 2005) and these were used as a starting point for the toxicity section. On behalf of Syngenta Crop Protection and Stauffer Management Company, Integral Consulting recently reviewed the toxicity of α-, β- and γ-HCH with published literature until March 2011 (Integral Consulting, 2011a, b, c). Literature searches were conducted to identify any further relevant papers that were not considered in those publications until October 2012.

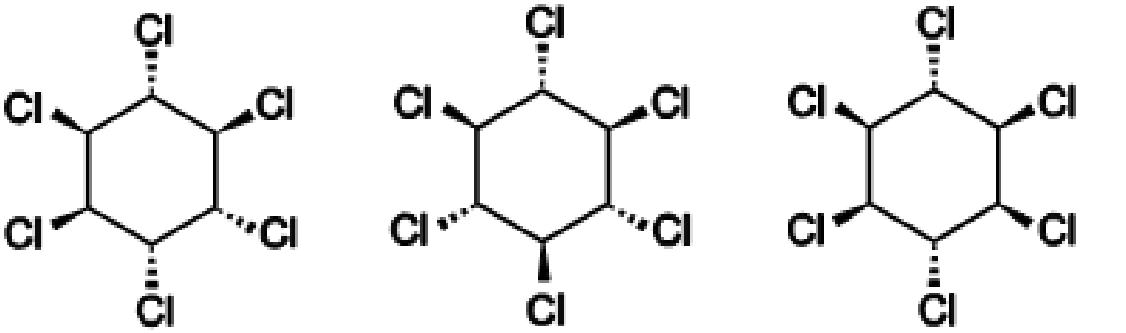
General background on HCHs

4. HCHs are organochlorines and encompass a group of eight isomers; α-, β-, δ-, ε-, ζ-, η-, θ- and γ-HCH. Technical-grade HCH consists of approximately 60–70% α-HCH, 5–12% β-HCH, 10– 15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH (Kutz et

al. 1991). This review focuses on α- β- and γ-HCH since these are listed as persistent organic pollutants in the Stockholm convention. Their structures are presented in Figure 1.

Figure 1. Chemical structures of α-, β-, and γ-HCH

α-HCH (CAS 319-84-6) β-HCH (CAS 319-85-7) γ-HCH (CAS 58-89-9)



5. Because of their lipophilic properties and persistence in the environment, β-HCH, followed by α-HCH and to a lesser extent γ-HCH bioaccumulate and biomagnify in the food chain. Their semivolatility allows them to be transported long-range and undertake “cold condensation”, thus increased levels are usually found in water in colder regions. The long-range atmospheric transport has resulted in global distribution especially of the most stable isomers α- and β-HCH (EFSA, 2005).

6. The current maximum levels in force for pesticides are described in Directive 2006/141/EC on infant formula and follow-on formula, and Directive 2006/125/EC on processed cereal foods and baby foods for infants and young children. The maximum pesticide level for other food products is described in Directive 2005/396/EC. A general maximum residue level (MRL) (0.01 mg/kg) is applicable ‘by default’ in all cases where an MRL has not been specifically set for a product or product type. This is the case for infant and follow-on formula and processed cereal foods and baby foods for infant and young children. In relation to other food products for human consumption, several different MRLs apply depending on the HCH isomer and the food commodity although for the majority of them the default MRL applies. MRLs range from 0.004 to 0.2 mg/kg for α-HCH, from 0.1 to 0.003 mg/kg for β-HCH, and from 0.001 to 1 mg/kg for γ-HCH (Directive 2013/212/EC).

α-HCH

Toxicokinetics

7. From the limited available data it appears that α-HCH is almost completely absorbed from the gastrointestinal tract (WHO-IPCS, 1992).

8. Following absorption α-HCH is predominantly distributed to the liver, kidney, brain, muscle and adipose tissue with marked accumulation in the fat (WHO-IPCS, 1992).

9. The metabolism of α-HCH involves dechlorination. The major phase I metabolite of α-HCH is 2,4,6- trichlorophenol, with lesser amounts of 1,2,4-, 2,3,4-

and 2,4,5-trichlorophenol, 2,3,4,5- and 2,3,4,6-tetrachlorophenols and 1,3,4,5,6-pentachlorocyclohex-1-ene. A glutathione conjugate has also been reported (WHO-IPCS, 1992).

10. After intraperitoneal injection to rats, 40-80% of α-HCH was excreted in the urine and 5-20% in the faeces. No studies have been found reporting plasma elimination half-life values for α-HCH in humans and rodents, or from which they could be adequately inferred. The half-life for clearance from the fat was reported to be 6.9 days in female rats and 1.6 days in male rats. The half-life for elimination from the brain of female rats was reported to be 6 days (WHO-IPCS, 1992).
Induction of drug metabolising enzymes

11. In the promotion study by Masuda et al, (2001) (see paragraph 25), rats were injected with a single dose of 200 mg/kg bw diethylnitrosamine (DEN) followed by 2 weeks of basal diet after which a diet containing α-HCH at 0.01, 0.1, 0.5, 1, 2, 4, 7.5, 15, 30, 60, 125 or 500 ppm was given for 6 weeks. A two thirds partial hepatectomy was carried out at the end of week 3. CYP3A2 protein expression was statistically significantly increased at dietary concentrations of 15 to 500 ppm. CYP2B1 protein protein expression was increased at 60 and 500 ppm (the only two doses investigated for this parameter).

12. Similarly, in a study by Puatanachokchai et al, (2006), rats were injected with 100 mg/kw bw DEN 3 times in one week followed by a diet containing 0.01, 0.05, 0.1, 1, 50 or 500 ppm for 10 weeks. At 50 and 500 ppm of α-HCH, CYP2C11/6, CYP2E1, CYP3A1/2 and NADPH-cytochrome P450 reductase protein expression was increased by more than 2 fold, 1.5 fold, 5 fold and more than 2.5 fold, respectively. CYP2B1 was not detected in the control and it increased up to 175 pmol/mg protein at 500 ppm.

Toxicity of α-HCH

13. α-HCH is of low acute toxicity, with oral LD50 values in the region of 1,000 – 4,000 and 500 to 5,000 mg/kg bw in mice and rats respectively. Signs of toxicity were mainly related to stimulation of the nervous system (WHO-IPCS, 1992).

14. Hepatotoxicity of α-HCH has been reported in many studies. For example, liver hypertrophy was reported at 10 mg/kg bw/day (reported by the authors to be equivalent to 0.5 mg/kg bw/day) in a 90-day study in rats given 2, 10, 50 and 250 mg/kg diet. The no observed adverse effect level (NOAEL) was 2 mg α-HCH/kg diet (equivalent to 0.1 mg/kg bw/day). Decreased body weight was reported at the highest dose (equivalent to 12.5 mg α-HCH/kg bw/day) (Kuiper et al., 1985; cited in EFSA, 2005).

15. α-HCH has been reported to be the most potent HCH isomer in inhibiting gamma-aminobutyric acid (GABA)-mediated chloride ion uptake in mouse brain, which is considered to play a primary role in its action on the central nervous system (CNS) (WHO-IPCS, 1992). However information on the dose response relationship for neurotoxicity following oral exposure is not available.

16. Signs of immunosuppression (reduced levels of immunoglobulins) were seen at 2.5 mg α-HCH/kg bw/day (Kuiper et al., 1985; cited in EFSA, 2005). No local lymph node assays (LLNA) or other studies investigating the allergenic potential of α-HCH have been found.

17. No studies investigating the potential reproductive toxicity of α-HCH have been found.

Genotoxicity

18. α-HCH was not mutagenic in a number of assays including the Ames test in *Salmonella typhymurium*, the reverse mutation assay in Escherichia coli and *Saccharomyces cerevisiae* and spot test with *Bacillus subtilis*.

19. Iverson et al, (1984) reported a low level of binding of [^{14}C]α-HCH to calf thymus DNA in the presence of liver microsomes from phenobarbitone-treated mice, but not from untreated mice. Binding to protein was two to three orders of magnitude higher than binding to DNA. In mice dosed with 25 mg/kg bw [^{14}C]α-HCH i.p., binding to DNA in the liver was reported at a level one to two orders of magnitude lower than binding to protein, but with no impact of pretreatment with phenobarbital. The authors concluded that these findings suggested that the tumorigenic response observed with α-HCH does not result from a genotoxic mechanism.

20. In a study by Sageldorff et al, (1983), male mice were dosed by oral gavage with 6.5 and 8.5 mg/kg [^{3}H]α-HCH and liver DNA was isolated to determine covalent binding. The authors reported that the low level of DNA binding of α-HCH did not correlate with susceptibility to tumour induction in three different mouse strains and was more than three orders of magnitude lower than would be expected if the mechanism of tumour induction was by genotoxicity mediated by DNA-binding e.g. for carcinogens like aflatoxin B1 or dimethylnitrosamine.

21. Kalantzi et al, (2004) reported an increase in micronuclei in human mammary carcinoma cells MCF-7 cells after treatment at pM levels, a finding that is implausible in view of the other data. These authors also reported that DNA breakage was statistically significantly increased compared to control in the Comet assay in MCF-7 with and without repair inhibitors at 100 µM α-HCH, the only concentration for which data were reported (Kalantzi et al, 2004). α-HCH in the range 56 to 320 µM produced a concentration-dependent increase of DNA strand breaks in the alkaline elution assay in rat hepatocytes and in hepatocytes in 4 out of 5 human donors but not in mouse hepatocytes (Mattioli et al, 1996).

22. Feeding of male rats for 3 weeks with 600 ppm α-HCH in the diet resulted in increased chromosomal abnormalities but not altered ploidy (Hitachi et al, 1975).

23. The COT considered that the available data suggest that α-HCH does not exert important genotoxicity.

Carcinogenicity

24. α-HCH has been shown to cause liver tumours in multiple mouse strains and in rats, with rats being less sensitive than mice (IPCS, 1992; Integral Consulting, 2011).

25. Results obtained from initiation-promotion studies for α-HCH support the role of α-HCH as a tumour promoter. In the study by Masuda et al, (2001), rats were injected with 200 mg/kg bw DEN followed by 2 weeks of basal diet after which they were fed diet containing α-HCH at 0.01 to 500 ppm for 6 weeks. A two thirds partial hepatectomy was carried out at the end of week 3. The numbers and areas of glutathione-S-transferase placental (GST-P)-positive foci were statistically significantly increased at levels from 2 and 7.5 ppm respectively, with a NOAEL of 1 ppm. Using the factor of 0.09 recommended by EFSA for converting feed concentration to dose in rats in subchronic studies (EFSA 2012), 1 ppm is equivalent to 0.09 mg/kg bw/day. In the study by Puatanachokchai et al, (2006), rats were injected 3 times in one week with 100 mg/kw bw DEN followed by a diet containing 0.01 to 500 ppm α-HCH for 10 weeks. The number of GST-P-positive foci was statistically significantly increased at 50 and 500 ppm and the area was also increased at 500 ppm. The authors reported these feed levels to be equivalent to 2.8 to 29.9 mg/kg bw/day. The NOAEL in this study was at the feed concentration of 1 ppm, reported by the authors to be equivalent to 0.055 mg/kg bw/day.

26. Overall, the COT concluded the evidence suggests that α-HCH is primarily a phenobarbitone-like inducer (i.e. induces CYP2B subfamily and other CYP enzymes) in the rat and hence will be an activator of the constitutive androstane receptor (CAR). Because the available studies do not provide convincing evidence of genotoxicity, and liver tumour formation in rodents is likely to be through a CAR activation mode of action (MOA), the carcinogenicity is unlikely to be relevant for humans.

Observations in humans

27. A hospital-based case-control study in India investigated associations between breast cancer and blood levels of α-HCH, as well as β- and γ- HCH and other organochlorine compounds (Mathur et al., 2002). The study included 135 breast cancer patients and 50 female hospital controls. All pesticide levels were higher in breast cancer patients than in controls. Age-stratified analyses were limited by small numbers in the control group, but found higher α-HCH blood levels in cases aged 41–50 years than in control women of the same age. However, no adjustment was made for potentially important confounding factors such as breast feeding, child-bearing, occupation and body fat. Nor did the analysis take into account the levels of other organochlorines. Therefore it is inconclusive with respect to associations with the HCHs.

Allergy

28. No studies have been found associating α-HCH and incidence of allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

29. Table 1 summarises the tolerable daily intakes (TDI) and RfDs that have been established or proposed for α-HCH.

Table 1 TDIs and RfD proposed for α-HCH.

Source of HBGV	HBGV µg/kg bw/day	Critical effect and species NOAEL in mg/kg bw/day	UF	Study selected to derive HBGV
Integral Consulting (2011a)	RfD 0.3	Hepatocarcinogenesis in male rats NOAEL 0.1	300	Masuda et al, 2001
Slooff and Matthijsen, (1988) confirmed by RIVM (2001)	TDI 1	Liver changes in rats NOAEL 0.1	100	Not identified
Health Canada (1992), cited in EFSA (2005)	Group TDI 0.3 for all HCH isomers	Unknown		Not identified

30. The RfD for α-HCH proposed by Integral Consulting (2011a) was based on the study of Masuda et al, (2001) on promotion of hepatocarcinogenicity in male rats (paragraph 25). The NOAEL was reported to be 0.1 mg/kg bw per day. An uncertainty factor (UF) of 300 (10 each to account for intra- and inter-species extrapolation, and 3 for database uncertainties) was applied, resulting in the proposed RfD of 0.3 µg/kg bw per day.

31. The RIVM TDI for α-HCH was originally established by Slooff and Mathijsen, (1988). The TDI was based on a 90 day oral study in rats with a NOAEL for liver changes (no further information given) equivalent to 0.1 mg/kg bw/day. Applying an UF of 100, the TDI was established at 1 µg/kg bw/day. No more information was provided on the original study or the rationale for applying only the default UF of 100 to the NOAEL. RIVM (2001) re-evaluated the scientific evidence and confirmed the previously established TDI. The basis for the Health Canada TDI is not publically available.

32. The COT concluded that it was not possible to endorse any of these values. The findings of Masuda et al. (2001) related to tumour promotion were considered to be of uncertain human relevance and there was insufficient information on the study used by RIVM (2001) as the basis for deriving a TDI. The COT concluded that the available information was insufficient to propose a TDI, and that it was more appropriate to apply a margin of exposure (MOE) approach using the NOAEL of 0.1 mg/kg bw/day for hepatotoxicity, supported by the findings in relation to tumour promotion in the study by Masuda et al. (2001). The toxicity of α-HCH has not been well characterised. In particular, there are no studies of reproductive toxicity. Taking into account that a UF of 100 is required for inter- and intra-species differences, and allowing an additional factor of 10 for the gaps in the database, the COT concluded that a MOE of 1,000 or greater compared to the NOAEL of 0.1 mg/kg bw/day would not be a health concern.

Sources of α-HCH and occurrence levels

Drinking water

33. Reports from water companies across the UK provide the results of analyses of α-HCH. For example, in the data summary tables from 2011 (published by the Drinking Water Inspectorate12th July 2013) it was reported that the 99th percentile for α-HCH was < 0.002 µg/L for 1172 samples taken from the following areas: Wales, Trent-Severn, Bristol and Wessex (Dŵr Cymru Welsh Water, 2012; Severn Trent Water Ltd, 2012; Bristol Water Plc, 2012; Wessex Water Services Ltd, 2012).

Breast milk

34. In a study that included 92 samples from 48 donors in the UK sampled in 2001-2002, α-HCH was not detected at a limit of detection of 10 µg/kg fat (Wooldridge et al, 2004).

35. In the context of the 3rd WHO human milk field study (2000-2001) α-HCH was analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine) and 11 pools from 6 non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA) (Malisch et al., 2004). The α-HCH concentrations in the pools from Bulgaria, Russia and Ukraine ranged from 2 to 6 µg/kg fat with the highest value from the Ukraine. These samples were possibly affected by local sources of contamination not representative of the UK. α-HCH was not detected in other European samples at a limit of detection of 1 µg/kg fat.

Infant formula

36. Infant formulae are included in the UK national monitoring programme for pesticide residues in food, which is overseen by the Defra Expert Committee on Pesticide Residues in Food (PRiF). Infant formula was last surveyed in July-September 2009 and was not detected at or above the reporting limit[1] of 10 µg/kg, which corresponds to the current MRL (PRiF, 2010).

37. A study from 2001 to 2006 on marketed food including infant formula in Barcelona (Catalonia, Spain) did not find any sample containing α-HCH at or above the quantification limits out of the 1484 samples analysed (Fontcuberta et al., 2008). The quantification limits were 10 µg/kg in low fat food and 5 µg/kg in high fat food.

38. Recent monitoring of the wider UK food supply, which has not identified residues of α-HCH in whole milk at a reporting limit of 0.002 mg/kg, indicates that levels in cows' milk-based infant formulas are likely to be well below the MRL and reporting limit for infant formula of 0.01 mg/kg. Soya milk was last included in the UK pesticide monitoring programme in 2006. α-HCH was not identified at a reporting

[1] The reporting limit is defined as the lowest calibrated level employed during analysis for a pesticide residue, and so quantification of lower levels would be unreliable.

limit of 0.002 mg/kg, nor was it detected in soya pieces or tofu at this reporting limit. This indicates that levels in soy-based infant formula are likely to be well below 0.01 mg/kg.

Weaning diet

39. Cereal-based, fruit and vegetables, and other products containing egg, fish, meat or cheese to be consumed by infants were last surveyed by PRiF in March (2011) (PRiF, 2011), August (2011) (PRiF, 2012) and February (2009) (PRiF, 2009) respectively. No α-HCH was detected at or above the reporting limits of 10 µg/kg each.

40. Recent monitoring by PRiF of the wider UK food supply has not identified residues of α-HCH, including in fatty foods, which would be expected to contain the highest levels. For example, in the 2012 UK pesticide monitoring programme, α-HCH was not identified in whole milk, lamb, eggs or butter at reporting limits of 0.002 mg/kg each, or in cheese at a reporting limit of 0.01 mg/kg. It therefore appears very unlikely that α-HCH would be present in composite food products such as infant foods at levels close to the MRL and reporting limit of 0.01 mg/kg.

Exposure

41. An on-going market basket study performed between 1994 and 2003 in the Czech Republic, where HCHs were produced and used for a long time, indicated a decline of daily dietary intakes. The median daily intake for α-HCH in 1994 was 4.3 ng/kg bw (Ruprich et al., 1995) and the corresponding intake reported in 2002 was 1.6 ng/kg bw (Ruprich et al., 2003).

42. Biomonitoring data in Germany indicate a decrease in exposure to α-HCH. The third German Environmental Survey conducted in 1998 (GerES III) in blood samples from 4800 subjects geographically representative for the German population, age (18 to 69 years) and gender (Becker et al, 2002). The arithmetic mean and the maximum value for all subjects was <0.1 and 0.4 µg/L respectively. The limit of quantification (0.1 µg/L) was exceeded by 1.7% of the subjects. The subsequent survey (GerES IV) carried out between 2003-2006 in blood samples from 1063 children aged 7 to 14 from 150 randomly selected locations in Germany reported no subjects above the level of quantification of 0.016 µg/L (Schulz et al, 2009).

43. The consumption values used for the exposure calculation for breast milk and infant formula were 800 mL (average consumer) and 1200 mL (high consumer) as proposed by EFSA (2012) and applied in other COT papers. In its dietary exposure estimations, the COT has previously used bodyweight data from a relatively old survey (DH, 1994). Bodyweight data are now available from the recently published UK Dietary and Nutrition Survey of Infants and Young Children (DNSIYC) (DH,

2013), with average bodyweights of 7.8, 8.7 and 9.6 kg for infants aged >4.0-6.0, >6.0-9.0 and >9.0-12.0 months old, respectively. Since DNSIYC did not include infants younger than 4 months, in this statement a value of 5.9 kg for infants aged 0-3 months from an earlier survey (DH, 1994), is assumed for infants aged 0-4.0 months.

Breast milk

44. Since there are no quantified data for occurrence of α-HCH of relevance to breast milk in the UK, a worst case estimation has been based on the limit of detection (< 1 µg/kg fat) for most European countries within the 3rd WHO human milk field study, which were considered to most likely be relevant for the UK. The exposures estimated are presented in Table 2, calculated on the assumption that the fat content of breast milk was 3.5 %.

Table 2. Theoretical maximum exposure of infants to α-HCH for average and high consumption of breast milk.

	Exposure (µg/kg bw per day)			
	0 – 4 months	**>4.0 – 6.0 months**	**>6.0 – 9.0 months**	**>9.0 – 12.0 months**
Average	< 0.005	< 0.004	< 0.003	< 0.003
High level	< 0.007	< 0.005	< 0.005	< 0.004

45. Two studies carried out in the Czech Republic with samples in the years 1994 and 2002 reported a reduced estimated exposure from 0.004 to 0.002 µg/kg bw/day (Ruprich et al, 1995; 2003).

Infant formula

46. In the surveys carried out in the UK by PRiF on infant formula no samples were found at or above the MRL (10 µg/kg) for α-HCH. Following the recommendations of the Office of Pesticides Programme (OPP) within the US Environmental Protection Agency (US EPA), the exposure has been estimated using ½ MRL (5 µg/kg) (US EPA, 2000). From the summary reports provided by the water companies in several regions in the UK the 99th percentile for α-HCH was < 0.002 µg/L. If α-HCH isomer was present at 0.002 µg/L in water used to reconstitute infant formula, the exposure from the water would be 0.002 µg, equivalent to 0.0003 µg/kg bw day for infants younger than 4 months with an average weight of 5.9 kg. This would have a negligible impact on the total from reconstituted formula, if the level in the formula was ½MRL. Table 3 provides the exposure that would result from consumption of infant formula containing α-HCH at ½ MRL. It is likely that actual exposure would be much lower than this.

Table 3. Estimated exposure of infants to α-HCH from average and high consumption of infant formula assuming it contains α-HCH at 50% of the MRL

	Maximum exposure (µg/kg bw per day)

Consumption	0 – 4 months	>4.0 – 6.0 months	>6.0 – 9.0 months	>9.0 – 12.0 months
Average (800 ml)	0.09	0.07	0.06	0.06
High level (1200 ml)	0.14	0.10	0.09	0.08

It is assumed that 0.135 kg of powder is used to reconstitute 1 L of formula, as recommended by the Center for the Evaluation of Risks to Human Reproduction (CERHR), which is consistent with infant formula manufacturers' advice in the UK. *The contribution from water used for reconstitution has not been added as it is likely to make a negligible contribution to total α-HCH exposure from infant formula.

Weaning diet

47. The average and 97.5th percentile of total solids consumed by infants from the DNSIYC is estimated at 35 and 54 g/kg bw/day respectively. No samples were found at or above the MRL (10 µg/kg) for α-HCH in the surveys carried out in the UK on cereal-based, fruit and vegetables, and other products containing egg, fish, meat or cheese to be consumed by infants. Thus, the maximum exposure to α-HCH isomer for average and high-level consumption based on ½ MRL (paragraph 46) is less than 0.18 and 0.27 µg/kg bw/day respectively. It is likely that actual exposure from solid infant foods would be much lower than this.

Risk characterisation

48. The MOEs calculated for maximal predicted exposure to α-HCH compared to the reference point of 0.1 mg/kg bw/day are shown in Table 4. The MOEs for maximal exposure from breast milk, of less than 0.007 µg/kg bw/day, exceed 14,500 and are not a concern.

49. The MOEs calculated for predicted exposure from infant formula for average and high consumers are > 1100 and > 740. The youngest age group of high consumers is below the value of 1,000 identified by COT as of no health concern; however, high consumers would gradually be at levels above MOE. All other combinations of age groups and consumers are above the established MOE. The available data indicate that exposure from water used to reconstitute infant formula would be negligible.

50. The MOEs calculated for exposure to α-HCH from solid infant food for average and high consumers are > 600 and > 400 respectively. Again these MOEs are below the value of 1,000 identified by COT as of no health concern, but actual exposures could be much lower since they are based on non-quantified data and the occurrence levels in ingredients used to reconstitute infant formula and infant food products are substantially below ½ MRL.

Table 4. MOEs (rounded) calculated based on potential maximum/estimated exposure of infants to α-HCH from average and high consumption breast milk, infant formula and infant foods

Isomer	Consumption	Exposure (µg/kg bw/day)			
		0 - 4.0	>4.0 – 6.0	>6.0 – 9.0	>9.0 –

		months	**months**	**months**	**12.0 months**
Breast milk	Average	20,000	25,000	33,500	33,500
	High level	14,500	20,000	20,000	25,000
Infant formula (assuming compliance with EU legislation)	Average	> 1100	> 1400	> 1700	> 1800
	High level	> 740	> 1000	> 1100	> 1200
Infant food (assuming compliance with EU legislation)	Average	N/A	N/A	> 600	
	High level	N/A	N/A	> 400	

β-HCH

Toxicokinetics

51. From the limited available data it appears that β-HCH is almost completely absorbed from the gastrointestinal tract (WHO-IPCS, 1992).

52. Following absorption β-HCH is predominantly distributed to the liver, kidney, brain, muscle and adipose tissue with marked accumulation in the fat. β-HCH is reported to pass the blood-brain barrier less readily than other HCH isomers. Transplacental and lactational transfer is also reported (WHO-IPCS, 1992).

53. The metabolism of β-HCH involves dechlorination. β-HCH is metabolised predominantly to 2,4,6- trichlorophenol, but with fewer other chlorophenols having been reported. Conjugation is reported to be mainly with glucuronide or sulphate (WHO-IPCS, 1992).

54. In rats, 70% of β-HCH is eliminated within 28 days, one third of this being excreted in the urine as metabolites, suggesting that faecal excretion is more important for β-HCH than for α-HCH. A 2-stage process has been reported for elimination of β-HCH in mice, the half-life for the first stage being 2.5 days and that for the second stage being 18 days. The half-life for clearance from blood in rats (sex not specified) was 1 month, and the half-life for clearance from fat was 14 days in male rats and 28 days in female rats. A half-life of 22 days for clearance from "internal organs" and 20 days for the brain were reported in female rats. (WHO-IPCS, 1992).

55. The elimination of β-HCH in humans was investigated by Jung et al., (1997) in a group of 40 former workers of a lindane-producing plant. Assuming a first-order kinetic model for excretion, the median half-life of β-HCH was 7.2 years calculated by concentrations in whole blood and 7.6 years calculated by concentrations in extractable lipids.

56. Breast milk is a route of excretion of β-HCH in lactating women. Waliszewski et al. (2009) determined the concentration of organochlorine pesticide levels in human breast milk samples from the 4th to the 30th day postpartum from 40 participants who had lived a minimum of 5 years in Veracruz (Mexico). The β-HCH residues presented as mean and standard deviation (SD) in the breast milk samples decreased during lactation from 95 (60) on the 4th day to 66 (45) µg/kg on the 30th day.

Induction of drug metabolising enzymes

57. There are few studies published on the effects of β-HCH on drug metabolising enzymes, which could be a reflection on their lack of relevance in the mode of action of β-HCH. A study by Van Velsen et al. (1986a), showed that β-HCH is a CYP inducer in the rat but due to techniques available at the time the CYP enzymes were not identified.

Toxicity of β-HCH

58. β-HCH is of low acute toxicity, with oral LD50 values > 8,000 mg/kg bw in rats and > 16,000 mg/kg bw in mice. Signs of toxicity were mainly related to stimulation of the nervous system (WHO-IPCS, 1992).

59. Hepatotoxicity has been reported for β-HCH in many studies. For example, toxicity was investigated in a 13-week study in rats with β-HCH at 0, 2, 10, 50, or 250 mg/kg feed. There was a statistically significant increase in liver weight from 10 mg/kg feed in both sexes. Hyalinization of centrilobular cells was observed in some males and females from 2 and 250 mg/kg feed onwards, respectively. CYP content was statistically significantly increased from 50 mg/kg feed in males, and in all female dose groups. Effects on the thymus, testes and ovaries, and severe morbidity were observed in the highest dose group (Van Velsen et al., 1986a). The LOAEL from this study was 2 mg/kg feed, the lowest feed concentration. This is equivalent to 0.18 mg/kg bw per day based on the EFSA (2012) default values for subchronic studies.

60. β-HCH has been observed in acute and semi-chronic studies to induce ataxia in rats with a NOAEL of 5 mg/kg bw per day and 19 mg/kg bw per day in mice. Other effects such as reduced tail nerve conduction velocity have been reported at higher concentrations (WHO-IPCS, 1992).

61. A study by Van Velsen et al., (1986a) on rats fed β-HCH in the diet for 13 weeks reported a number of immunological effects with a NOAEL at 50 mg/kg feed, equivalent to 4.5 mg/kg bw/day. The described effects were significantly decreased levels of red and white blood cells in conjunction with increased extramedullar haematopoiesis in the spleen and hypertrophy of the adrenal gland and atrophy of the thymus.

62. In a 2 generation-study by van Velsen, (1986b in PhD Thesis, cited in IPCS-WHO, 1992), male and female rats in the F0 generation were fed β-HCH in the diet at 2, 10 or 50 mg/kg for 13 weeks. Almost complete infertility was reported in the F1 generation for the group ingesting 50 mg/kg together with reduced litter size and death before weaning. In the group ingesting 10 mg/kg precocious vaginal opening and complete infertility in the second generation were reported. No effects were reported for the group on 2 mg/kg. A publication by van Velsen et al, (1986a) reported endocrine effects in the parental generation. In females, the weight of the adrenal gland was statistically significant increased at 2 and 10 mg/kg feed (absolute) and 250 mg/kg feed (absolute and relative). An increase in the uterus weight was reported at 10 mg/kg feed (absolute). Other reported effects were atrophy of the testes, reduced size of seminiferous tubules and lower number of Leydig cells were observed in males with a NOAEL of 50 mg/kg feed, equivalent to 4.5 mg/kg bw per day.

Genotoxicity

63. β-HCH was not mutagenic in the spot test in *Bacillus subtilis* (Tanooka et al, 1977). A study investigating DNA binding in male mice, an extremely low level of binding liver 10 h after oral administration of β-[^{3}H]HCH i.p. (Sagelsdorff et al, 1983).

Positive responses were reported in a micronucleus test in MCF-7 cells at 1 and 10 pM with the higher concentration providing smaller effects than the lower concentration, which suggests a chance observation at this very low concentration. In a Comet assay in MCF-7 cells, a weak positive was observed at 100 µM, the only concentration for which data were reported (Kalantzi et al, 2004). Overall, the COT considered that β-HCH is unlikely to exert important genotoxicity.

Carcinogenicity

64. Eight studies of carcinogenicity have been reported for β-HCH in rats and mice. While there are limitations in several of them only a study by Thorpe and Walker (1973) provided indications of carcinogenicity. In this study male and female mice were fed β-HCH at 200 ppm in the feed for 2 years (equivalent to 30 mg/kg bw/day applying the EFSA (2012) conversion factor of 0.15 for chronic mouse studies). There was decreased survival in treated animals compared to controls, liver enlargement after 50-60 weeks and ataxia before death. Mice dying early had hepatic and extra-hepatic tumours.

65. Overall, the COT concluded the available data provided conflicting evidence that β-HCH is carcinogenic in mice. The increased incidence of liver tumours in a single study (Thorpe and Walker, 1973) is considered not relevant for humans since the mechanism of action is CAR associated through CYP2B induction.

Observations in humans

66. In 2004, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) considered whether exposure to organochlorine insecticides including β-HCH and lindane was associated with an increased risk of breast cancer (COC, 2004). At that time, the COC concluded that:

"β-HCH should be regarded as having weak *in vivo* oestrogenic activity. There is evidence from investigations undertaken in the UK for a decline in β-HCH concentrations in human fat samples after 1982/3. The available epidemiological studies do not suggest any evidence for an association between β-HCH and increased risk of breast cancer. Overall the available data do not suggest that environmental exposure to β-HCH is a cause for concern as a risk factor for human breast cancer". Further studies have been published since 2004 until October 2010 focussing on the potential association between β-HCH and breast cancer. A study by Ociepa-Zawal et al, (2010) comparing levels of β-HCH in healthy subjects (n=23) and breast cancer patients (n=54) in Poland reported higher levels of β-HCH in adipose tissue in the latter population. Two studies carried in Japan did not find an association between serum plasma levels of β-HCH and breast cancer (Itoh et al, 2009; Iwasaki et al, 2008).

67. Most other studies investigating associations of β-HCH with risk of cancer have focused on non-Hodgkin lymphoma (NHL). Five case-control studies were found which looked at this. Cantor et al (2003) conducted a case-control study of 74 cases and 174 controls nested in a community-based US cohort. This had the strongest design of the five studies as serum samples were obtained well before

diagnosis (a median of 12 years). After adjustment for covariates including PCBs, there was no consistent trend in risk with increasing concentration of β-HCH, and risk in the highest quartile of concentrations was 1.5 (95% CI 0.5-4.3) in comparison with the lowest quartile – i.e. not statistically significant. A European case-control study with 174 cases and 203 controls (Cocco et al, 2008) and a US study with 100 cases and 100 controls (de Roos et al, 2005) found no association between NHL and higher β-HCH in plasma. A further US study by Quintana et al (2004) of 175 cases and 481 controls measured β-HCH in adipose samples from cadavers and surgical patients with a variety of diagnoses, and found that risk of NHL was elevated for the highest quartile of concentrations (OR 2.47, 95% CI 1.34-4.55). Although risks were lowest in the two middle quartiles of exposure, there was a highly significant trend in risk across the four exposure categories (P=0.0001). However, associations were attenuated after adjustment for other pesticides (heptachlor epoxide, p,p'-DDE and dieldrin) in two-pollutant statistical analyses, only remaining statistically significant after adjustment for one of these three (p,p'-DDE). A Canadian study by Spinelli et al, (2007) involving 422 cases and 460 controls found significant associations of β-HCH and five other pesticides with NHL (OR for highest quartile of β-HCH 1.59, 95% CI 1.01-2.49), but the study did not adjust for other pesticide residues or PCBs, with which β-HCH was moderately correlated.

68. Taken together, the inconsistent findings between studies are not strongly suggestive of increased risk of NHL in association with higher tissue concentrations of β-HCH. However, correlations of tissue concentrations with those of other persistent organic pollutants make it difficult to demonstrate associations with any single compound, and small numbers of subjects have limited the statistical power of most studies to explore associations with subtypes of NHL.

69. Three studies have evaluated associations between β-HCH and Parkinson's disease. Weisskopf et al (2010) reported a case-control study in Finland in which serum samples were taken before diagnosis and analysed contemporaneously. The study included 171 cases and 349 controls within a cohort of 40,221 individuals participating in the Finnish Mobile Clinic Health Examination Survey, who had provided serum samples during 1968-1972. Cases occurring up to 1994 were identified through a national registry and confirmed by neurologist review. The study found no associations between β-HCH and Parkinson's disease (although an association was seen with dieldrin). This contrasts with two case-control studies in which measurements were made after diagnosis and levels reported in blood were lower than those from the Finnish study. A US study comparing 149 cases and 134 controls (Richardson et al 2011) found that higher serum β-HCH was associated with increased risk of Parkinson's disease. This incorporated most (49 of 50 cases, 41 of 43 controls) subjects from an earlier report published by the same group (Richardson et al, 2009), which also indicated an association. Estimated ORs were 1.03 (95% CI 1.00 to 1.07) for an increase of 1ng/mg cholesterol, and 2.85 (95% CI 1.8-4.48) for levels above the detectable inter-quartile range. Petersen et al (2008) in a study of 79 cases and 154 controls in the Faroe islands also found that higher serum levels of β-HCH were associated with Parkinson's disease. None of the studies of Parkinson's disease adjusted for other pesticide exposures.

70. The epidemiological findings for Parkinson's disease are conflicting and therefore inconclusive. Notably, the largest study, which was least prone to bias

since samples were collected before diagnosis, did not find an association. No account was taken of potential weight loss related to illness, which may have affected the studies with samples taken after diagnosis.

71. Three studies have looked for, but not found, associations of β-HCH exposure with cryptorchidism. A nested case-control study (241 cases, 681 controls) by Pierik et al, (2007) found no evidence of a dose-response relationship between β-HCH in the serum of pregnant women and cryptorchidism in their sons, although two of the three highest of six quantiles (but not the highest quantile) showed significantly increased risks. Damgaard et al. (2006) did not find higher β-HCH levels in post-partum breast milk samples from mothers of 62 boys with cryptorchidism as compared with 68 controls. Hosie et al (2000) measured pesticide levels in fat samples from 18 cases and 30 controls, and there were no statistically significant associations with β-HCH.

Allergy

72. One relevant study has been found in the scientific literature when searching for β-HCH and incidence of allergy, atopic disease or hypersensitivity. A cross-sectional study by Miyake et al, (2011) investigated presence of β-HCH in breast milk and the incidence of allergic disorders in a Japanese population of women (n = 124). The definition of wheeze and asthma was based on criteria from the European Community Respiratory Health Survey whereas that of eczema and rhinoconjunctivitis was based on criteria from the International Study of Asthma and Allergies in Childhood. Adjustment was made for age, smoking, family history of allergic disorders, and education. The prevalence values of wheeze, asthma, eczema, and rhinoconjunctivitis in the past 12 months were 9.7%, 4.8%, 13.7%, and 29.8%, respectively. The median concentrations of β-HCH in breast milk were 28.3 ng/g lipid, respectively (range, 4.5-253 ng/g lipid, respectively). When the exposures were treated as continuous variables, no significant associations were found between concentrations of β-HCH and the prevalence of wheeze, asthma, eczema, or rhinoconjunctivitis. The results suggest that concentrations of β-HCH in breast milk are not evidently associated with the prevalence of wheeze, asthma, eczema, or rhinoconjunctivitis in young female Japanese adults (Miyake et al, 2011).

73. No other studies have been found associating β-HCH and incidence of allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

74. Table 5 summarises the tolerable daily intakes (TDI) and RfDs that have been established or proposed for β-HCH.

Table 5 TDIs and RfD proposed for β-HCH.

Source of HBGV	HBGV µg/kg bw/day	Critical effect and species NOAEL/LOAEL mg/kg bw/day	UF	Study selected to derive HBGV

Integral Consulting (2011b)	RfD 0.06	Hepatoxicity in rats LOAEL 0.18	3,000	Van Velsen et al, 1986a
Slooff and Matthijsen, (1988) confirmed by RIVM (2001)	TDI 0.02	Infertility in male rats NOAEL 0.02	1,000	Van Velsen et al, 1986b
Health Canada (1992), cited in EFSA (2005)	Group TDI 0.3	Unknown		Not identified

75. The RfD for β-HCH proposed by Integral Consulting, (2011b) was based on the study by Van Velsen et al, (1986a) on hepatotoxicity in rats exposed for 13 weeks, with a LOAEL of 0.18 mg/kg day (paragraph 59). The UF applied was 3,000 to account for inter- and intra-species differences, use of LOAEL, use of subchronic study, and database limitations, resulting in a proposed RfD of 0.06 µg/kg day.

76. The RIVM TDI for β-HCH was originally established by Slooff and Mathijsen, (1988). The TDI was based on a semi-chronic oral study on reproduction in rats reported in a PhD Thesis by Van Velsen (1986b) cited in WHO-IPCS (1992), with a NOAEL for infertility considered to be equivalent to 0.02 mg/kg bw/day (paragraph 59). Applying a UF of 1,000, the TDI established was 0.02 µg/kg bw/day. The basis for the UF of 1,000 was not stated. RIVM (2001) re-evaluated the scientific evidence and confirmed its TDI.

77. The COT concluded that it was not possible to endorse the HBGV proposed by RIVM due to insufficient information on the study. The COT concluded that the available information was insufficient to propose a TDI, and that it was more appropriate to apply a MOE approach using the LOAEL of 0.18 mg/kg bw/day based on centrilobular hypertrophy (paragraph 59) as a reference point. The toxicity of β-HCH has not been well characterised. The study on reproductive toxicity by Van Velsen (1986b) provides insufficient information. Taking into account that a UF of 100 is required for inter- and intra-species differences, and allowing additional factors of 3 for the absence of a NOAEL and 10 for the other gaps in the database. The COT concluded that a MOE of 3,000 or greater compared to the reference point of 0.18 mg/kg bw/day would not be a health concern.

Sources of β-HCH and occurrence levels

Drinking water

78. Reports from water companies across the UK provide the results of analyses of β-HCH. For example, in the data summary tables from 2011 (published by the Drinking Water Inspectorate12th July 2013) it was reported that the 99th percentile for α-HCH was < 0.002 µg/L for 566 samples taken from the following areas: Wales and Trent-Severn (Dŵr Cymru Welsh Water, 2012; Severn Trent Water Ltd, 2012).

Breast milk

79. A time-related decline in the levels of β-HCH in breast milk is apparent from the scientific literature. Table 6 shows the concentrations of β-HCH in breast milk from studies in UK populations published since 1965.

Table 6. UK studies measuring β-HCH in breast milk published since 1965.

N	µg/kg milk fat					Mean µg/kg whole milk	% samples with detectable residues	Years of sample collection	Reference
	A. mean	G. mean	Med.	Min.	Max.				
19	N.D.	N.D.	N.D.	7	33	13 (A or G not specified)	100	1963-1964	Egan et al, 1965
102	220	N.D.	150	10	4400	7	80	1979-1980	Collins et al, 1982
-	-	-	-	-	-	5 (A or G not specified)	95	1984	Ministry of Agriculture, Fisheries and food, 1998
193	80	N.D.	60	<20	990	2	82	1989-1991	Dwarka et al, 1995
156	68 (A or G not specified)		50	<8	750	1	36	1997-1998	Harris et al, 1999
54	40	15	17	1.2	1500	N.D.	100	2001-2003	Kalantzi et al, 2004

A. mean. Arithmetic mean, G. mean. Geometric mean, Med. Median, Min. Minimum, Max. Maximum., N.D. No data.

80. In a study that included 92 samples from 48 donors in the UK sampled in 2001-2002 , β-HCH was not detected in any sample at a limit of detection of 100 µg/kg fat (Wooldridge et al, 2004).

81. In the context of the 3rd WHO human milk field study (2000-2001) β-HCH was analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine) and 11 pools from 6 non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA) (Malisch et al., 2004). The β-HCH concentrations in the pools from European countries ranged from 11 to 279 µg/kg.

82. The occurrence levels of β-HCH in breast milk in studies on populations in the United States and Europe published since 1994 are shown in Table 7.

Table 7. US and European studies measuring β-HCH in breast milk published since 1994 in chronological order of sample collection.

Country (City/Region)	µg/kg milk fat median/mean/level as indicated (high percentile as indicated) (time data provided)	Years samples collected	Reference
Italy (average of Rome, Milan, Florence and Pavia)	130 mean **(not indicated)**	1987	Larsen et al, 1994
Germany (North)	200 median **(not indicated)** (1986)	1986-1997	Schade et al, 1998
	50 median **(not indicated)** (1997)		
Spain	240 unknown mean or median **(not indicated)**	1991	Hernandez et al. in Wong (2002)
Germany (Saxony)	40 median **(95th%, 7,970)**	1992-1993	Raum et al, 1998
Germany (Saxony)	59 median **(not indicated)**	1992-1993	Schlaud et al, 1995
German (Saxony – Rural areas)	45 median **(not indicated)**	1992-1993	Schlaud et al, 1995
Russia (Murmansk)	853 mean **(not indicated)**	1993	Polder et al, 1998
Russia (Monchegorsk)	740 mean **(not indicated)**	1993	Polder et al, 1998
Ukraine	731 median **(90th%, 1,305)**	1993-1994	Gladen et al, 1999
Norway (Oslo)	14 mean **(not indicated)**	2000-2001	Polder et al, 2008
Norway (Tromsø)	10 mean **(not indicated)**	2000-2001	Polder et al, 2008
Germany (North Ryne-Westphalia)	130 mean **(not indicated)**	1984	P Fürst, personal communication to EFSA, 2005.
	20 mean (**not indicated)**	2001	
North Germany	11.6 median **(unknown)**	2006	Zietz et al, 2008
USA (California)	0.22 urban median **(75%, 0.24)**	2002-2007	Weldon et al, 2011
	0.44 rural median **(75%, 0.52)**		

83. In a study on a German cohort, the median levels of β-HCH in breast milk were positively correlated with maternal age and negatively associated with parity and the total period of breast-feeding. Post-pregnancy body mass index (BMI) was a significant predictor of the likelihood of having higher concentrations of β-HCH in breast milk. Women who had followed a low-fat diet for at least 3 years had lower β-HCH levels in their breast milk than women whose diet included large quantities of meat (Schade, 1998).

Infant formula

84. Infant formula was last surveyed in the UK national monitoring programme for pesticide residues in food in July-September 2009 (PRiF, 2010) and was not detected at or above the reporting limits of 10 µg/kg each, i.e the current MRL.

85. A study from 2001 to 2006 on marketed food including infant formula in Barcelona (Catalonia, Spain) did not find any sample at or above the quantification limits out of the 1484 samples analysed (Fontcuberta et al., 2008). The quantification limits were 10 µg/kg in low fat food and 5 µg/kg in high fat food.

86. Recent monitoring of the wider UK food supply, which has not identified residues of β-HCH in whole milk at a reporting limit of 0.002 mg/kg, indicates that levels in cows' milk-based infant formulas are likely to be well below the MRL and reporting limit for infant formula of 0.01 mg/kg. Soya milk was last included in the UK pesticide monitoring programme in 2006. β-HCH was not identified at a reporting limit of 0.002 mg/kg, nor was it detected in soya pieces or tofu at this reporting limit. This indicates that levels in soy-based infant formula are likely to be well below 0.01 mg/kg.

Weaning diet

87. Cereal-based, fruit and vegetables, and other products containing egg, fish, meat or cheese to be consumed by infants were last surveyed by PRiF in March (2011) (PRiF, 2011), August (2011) (PRiF, 2012) and February (2009) (PRiF, 2009) respectively. No β-HCH was detected at or above the reporting limits of 10 µg/kg each.

88. Recent monitoring of the wider UK food supply has not identified residues of β-HCH, including in fatty foods, which would be expected to contain the highest levels. For example, in the 2012 UK pesticide monitoring programme, β-HCH was not identified in whole milk, lamb, eggs or butter at reporting limits of 0.002 mg/kg each, or in cheese at a reporting limit of 0.01 mg/kg. It therefore appears very unlikely that β-HCH would be present in composite food products such as infant foods at levels close to the MRL and reporting limit of 0.01 mg/kg.

Exposure

89. An on-going market basket study performed between 1994 and 2003 in the Czech Republic, where HCHs were produced and used for a long time, indicated a decline of dietary exposure. The median daily intake for β-HCH in 1994 was 8.4 ng/kg bw (Ruprich et al., 1995) and the corresponding intake reported in 2002 was 2.1 ng/kg bw (Ruprich et al., 2003).

90. Biomonitoring data in Germany also indicate a decrease in exposure to β-HCH. The third German Environmental Survey conducted in 1998 (GerES III) in blood samples from 4800 subjects geographically representative for the German population, age (18 to 69 years) and gender (Becker et al, 2002). The arithmetic mean and the maximum value for all subjects was 0.16 and 7.8 µg/L respectively. The limit of quantification (0.1 µg/L) was exceeded by 34% of the subjects. The subsequent survey (GerES IV) carried out between 2003-2006 in blood samples from 1063 children aged 7 to 14 from 150 randomly selected locations in Germany reported 76% of the subjects above the level of quantification of 0.04 µg/L with 0.01 and 0.1 µg/L as 50% and 95% percentile respectively (Schulz et al, 2009).

91. The values for consumption and body weight used for the estimation of exposure for β-HCH are as described for α-HCH in paragraph 43.

Breast milk

92. The occurrence value selected for the calculation of exposure to β-HCH was the mean value from the most recent UK study presented in Table 2, i.e. 15 µg/kg for β-HCH (Kalantzi et al., 2004). The mean, rather than the maximum value was selected because the distribution of the data and comparison with other studies indicated that the maximum value might not be reliable, and also because levels would have been expected to decrease since the milk was sampled by Kalantzi et al. (2001-2003). The exposures estimated are presented in Table 8, calculated on the assumption that the fat content of breast milk was 3.5 %.

Table 8. Estimated exposure of infants to β-HCH for average and high consumption of breast milk.

	Exposure (µg/kg bw per day)			
Consumption	**0 – 4.0 months**	**>4.0 – 6.0 months**	**>6.0 – 9.0 months**	**>9.0 – 12.0 months**
Average	0.07	0.05	0.05	0.04
High level	0.11	0.08	0.07	0.06

93. EFSA calculated, assuming an average intake of 800 ml breast milk with a fat content of 3.5 % that the concentration of 20 µg β-HCH/kg fat identified from a study in Western Germany in 1984 would result in a daily intake of 0.11 µg/kg bw for a fully breastfed infant weighing 5 kg (EFSA, 2005).

94. Two studies carried out in the Czech Republic with samples collected in the years 1994 and 2002 reported a reduced estimated exposure from 0.019 to 0.006 µg/kg bw/day (Ruprich et al, 1995; 2003). Mean exposure levels from samples collected in Spain between 1989 and 1990 were 0.27 and 0.23 µg/kg bw/day for a 1 and 3 months old respectively (Martinez Montero et al, 1993). Mean exposure levels from samples collected in Canada between 1993 and 1996 were 0.001 µg/kg bw/day (Stockholm Convention, 2007).

Infant formula

95. No samples were found at or above the MRL (10 µg/kg) for β-HCH in the surveys carried out in the UK by PRiF on infant formula. As for α-HCH, ½ MRL was used to estimate the exposure of infants to β-HCHs. The summary reports of water companies in several regions in the UK provided a 99th percentile for β-HCH < 0.002 µg/L equal to the occurrence level reported for α-HCH. Given the identical occurrence, the same exposure of infants for α-HCH applies to β-HCH (paragraph 46 and Table 3).

Weaning diet

96. The exposure estimates calculated from solid infant foods for α-HCH also apply to β-HCH given the selection of ½ MRL common to both isomers (paragraph 47).

Risk characterisation

97. The MOEs calculated for predicted exposure to β-HCH compared to the reference point of 0.18 mg/kg bw/day are shown in Table 9. Based on the available information, it is not possible to conclude that the MOEs are greater than 3000. The MOE for breastfeeding infants is below or equal to 3,000 for high consumers in all age groups and only below in the youngest group in average consumers.

98. The MOEs calculated for predicted exposure from infant formula for average and high consumers are > 2,000 and > 1,300 with only the two oldest groups (> 6.0 – 9.0 and > 9.0 -12.0) for average consumers with MOEs > 3,000. The available data indicate that exposure from water used to reconstitute infant formula would be negligible.

99. The MOEs calculated for exposure to β-HCH from solid infant food for average and high consumers are > 1000 and > 650 respectively. Again these MOEs are below the value of 3,000 identified by COT as of no health concern but actual exposures might be much lower since exposure estimates are based on non-quantified data and the occurrence levels in ingredients used to reconstitute infant formula and infant food products are substantially below ½ MRL.

Table 9. MOEs (rounded) calculated based on potential maximum/estimated exposure of infants to β-HCH from average and high consumption breast milk, infant formula and infant foods compared to the reference point of 0.18 mg/kg bw/day

Isomer	Consumption	0 – 4.0 months	>4.0 – 6.0 months	>6.0 – 9.0 months	>9.0 – 12.0 months
Breast milk alone	Average	2,600	3,600	3,600	4,500
	High level	1,600	2,300	2,600	3,000
Infant formula (assuming compliance with EU legislation)	Average	> 2000	> 2600	> 3000	> 3200
	High level	> 1300	> 1800	> 1900	> 2200
Infant food (assuming compliance with EU legislation)	Average	N/A	N/A	> 1000	
	High level	N/A	N/A	> 650	

γ-HCH

100. The term “lindane” commonly refers to pesticidal products that contain >99% γ-HCH, although it has also been used colloquially as a synonym for γ-HCH. In this statement the term “lindane” is used when referring to the product, and γ-HCH is used when referring to the chemical. Lindane has been used for topical insecticide treatment in humans and animals. It has been estimated that for each ton of lindane

produced, around 6-10 tons of other isomers, were generated (Stockholm Convention, 2009).

101. Pesticidal use of HCH products that contained less than 99.0 % γ-HCH was banned in the EU by Council Directive 79/117/EEC of 21 December 1978. The authorization for lindane use as a pesticide was withdrawn in the EU by Commission Decision 2000/801/EC of 20 December 2000. This action was taken primarily due to concerns with regard to its safety, in particular for operator exposure, the fate and behaviour of lindane in the environment and effects on non-target organisms.

102. There are no current Marketing Authorisations (product licences) in the UK for any medicines containing lindane as the active ingredient (Medicines and Healthcare products Regulatory Agency, personal communication, 2013). However, it is still used in other countries. For example, shampoos with lindane for medical purposes are approved by the US Food and Drug Agency (USFDA) and are marketed in the US. Lindane is typically used at 1 % concentration (Reynolds, 1996). USFDA suggests their use as a second-line medication for patients with scabies and lice who have failed or cannot tolerate first-line therapies (USFDA, 2007).

Toxicokinetics

103. γ-HCH is rapidly and extensively absorbed in mice and rats after oral dosing. Absorption is rapid with peak levels in blood after single administration of 20 mg/kg bw at 40 minutes to 5 hours after which a plateau was observed (FAO/WHO, 2002). Absorption through skin was shown in human volunteers with different solvents such as white spirit and alcohol (Dick et al., 1997).

104. The metabolism of γ-HCH is extensive in mammals, involving stepwise dehydrogenation, dechlorination and dehydrochlorination, which may be followed by conjugation with sulphate or glucuronide. CYPs appears to be involved in the phase I metabolism. The predominant metabolite is again 2,4,6-trichlorophenol, with varying amounts of other chlorophenols, depending on species. EFSA (2005) noted that 70 metabolites of γ-HCH have been identified in animals and humans, including (in no particular order) pentachlorophenol, 2,3,4,6- and 2,3,5,6-tetrachlorophenol and 2,4,6-trichlorophenol, tetrachlorophenols, 2,3,4,5,6-pentachlorobenzene and pentachlorocyclohexene and conjugates with glutathione, glucuronide and sulphate.

105. γ-HCH is extensively distributed throughout the body of rodents. In mice and rats, results from several studies showed similar distribution patterns with radiolabel detected in fat, brain, kidney, muscle, liver and ovary tissue after administration of lindane in the diet. The highest concentration was found in adipose tissue (FAO/WHO, 2002).

106. Urine is the major route of excretion of metabolites, with a smaller proportion in the faeces. The half-life of γ-HCH in rats was estimated to be 3–5 days, approximately 80% of the administered dose being excreted within 8 days (FAO/WHO, 2002).

Induction of drug metabolising enzymes

107. Parmar et al. (2003) reported dose-dependent increased levels of CYP 1A1/1A2, 2B1/2B2 and 2E1 enzymes in the liver and brain of rats dosed with γ-HCH at 2.5 mg/kg bw per day or more for 21 days. The induction of CYP2B in rat has been also confirmed by Matsuura et al. (2005).

Toxicity of γ-HCH

108. Oral LD50 for γ-HCH values are 56 to 250 mg/kg bw in mice and 140–190 mg/kg bw in rats (FAO/WHO, 2002).

109. In rats, γ-HCH exhibits renal toxicity that is considered not to be relevant to humans since it is a consequence of accumulation of α-2micro-globulin, a protein that is not found in humans (FAO/WHO, 2002).

110. Hepatocellular hypertrophy was observed in a number of studies of γ-HCH in mice, rats and rabbits. In a 2-year study of toxicity and carcinogenicity in rats, the NOAEL was 10 ppm in the diet (estimated by authors to be equal to 0.47 mg/kg bw per day) on the basis of increased liver weight, hepatocellular hypertrophy, increased spleen weight and deaths at 100 ppm (equal to 4.7 mg/kg bw per day) (Amyes et al., 1990; FAO/WHO, 2002).

111. There are a large number of studies on neurotoxicity of γ-HCH, which have been evaluated by JMPR (FAO/WHO, 2002). With a single exposure in rats, a NOAEL at 6 mg/kg bw was reported on the basis of increased fore-limb grip strength and decreased grooming behaviour. In a 90-day study in rats, the NOAEL for males was 7.1 mg/kg bw per day on the basis of hypersensitivity to touch and hunched posture. In a study of developmental neurotoxicity in rats, the NOAEL for maternal toxicity after 25 days of exposure *ad libitum* was 4.2 mg/kg bw per day on the basis of decreased body weight, decreased food consumption and increased reactivity to handling.

112. Based on a study in which mice were given γ-HCH at 0, 10, 40 or 160 mg/kg in the diet for 39 weeks to examine the effects on the total number of leukocytes and on the relative proportion of lymphocyte populations, JMPR concluded that γ-HCH is not immunotoxic (FAO/WHO, 2002).

113. Meera et al. (1992) investigated a number of different immunological endpoints in female rats exposed for 24 weeks to γ-HCH. Lymphocyte transformation and haemolytic plaque forming cell assay was observed at 0.012 mg/kg bw per day, the lowest dose tested. The lymphocyte proliferation response to concanavalin A increased up to 8 weeks with a later decrease after 12 weeks. An increase in the plaque forming assay number up to 8 weeks was followed by suppression up to 24 weeks. Other effects were reported at higher doses such as necrosis of thymus, lymph nodes and spleen.

114. Endocrine effects have been investigated in a number of studies. The most prominent effects are a decrease in the levels of thyroid hormones and modulation of luteinizing and follicle-stimulating hormone which are likely to be related with the

alteration of oestrous cycle in females. In male rats, exposure to γ-HCH led to a decrease in serum testosterone levels, epididymal sperm count, sperm motility and an increase in the percentage of abnormal sperm (Prasad et al, 1995) whilst in mice a reduction of primordial germ cells was reported in vivo (exposure during pregnancy) and in vitro by measuring the apoptosis rate (La Sala et al, 2009)..

115. There are a large number of studies that have investigated the reproductive toxicity of γ-HCH. The outcomes are diverse and occasionally inconsistent between studies. In rats, effects such as delayed vaginal opening, decreased ovary weight and decreased number of fetuses have been reported. In the F1 generation, the effects observed were decreased weight and viability of pups and increased spleen weight. Semi-chronic studies in mink ranging from 12 to 17 weeks exposure at 1 mg/kg per day induced effects such as reduced litter size (F2), reduced testis size (F3), reduced mating receptivity and increased embryo loss.

116. In an extended two-generation reproduction study (Matsuura et al., 2005), rats were exposed to γ-HCH at 10, 60 or 300 ppm diet for 10 weeks before and through mating until terminal necropsy (males); and through mating, gestation, lactation until F1 weaning at post-partum day 21 (females); the F1 were treated in the same manner as F0 animals after weaning at postpartum day 21. Findings in all generations included decreased body weight gain, increased liver weights and centrilobular hypertrophy and induction of hepatic drug metabolising enzymes. Reproductive effects included lack of nursing and retrieval behaviour, possibly due to effects on the nervous system, but no effects on oestrus cycle, spermatogenesis, mating, fertility, pregnancy or parturition. No changes were found in endpoints for endocrine disrupting activity. In the F0 males of the low dose group (with dose calculated by the authors equal to an average of 0.56 mg/kg bw/day) the relative liver weight was statistically significantly higher than control, but without clear dose-dependency. Other effects were observed at the high, and in some instances also in the mid dose group. Overall, the results of this study indicate a LOAEL of 0.6 mg/kg bw/day.

Genotoxicity

117. JMPR reviewed a large number of studies of γ-HCH (lindane), including assays for bacterial and mammalian cell mutation, chromosomal changes, DNA repair in vitro, and chromosomal aberration, sister chromatid exchange and dominant lethal mutations in vivo, and concluded that genotoxicity of lindane was found only at cytotoxic concentrations or in the presence of lindane precipitate and that lindane is not genotoxic (FAO/WHO, 2002).

118. Since the JMPR evaluation, Kalantzi et al, (2004) reported a weak positive in DNA breakage in the Comet assay in MCF-7 cells at a concentration of 100 µM γ-HCH in the presence of DNA repair inhibitors. An increase in micronuclei, doubling the number in the negative control, was reported in MCF-7 and human prostate cancer cell line (PC-3) at pM levels. It cannot be excluded that the positive response in the micronuclei test might be due to experimental error at such low concentrations. Since these results are not consistent with the earlier studies COT did not consider them convincing evidence of genotoxicity of γ-HCH.

Carcinogenicity

119. JMPR noted that lindane did not induce a carcinogenic response in rats, but increased incidences of adenomas and carcinomas of the liver were observed in agouti and pseudoagouti mice in a study of the role of genetic background in the latency and incidence of tumorigenesis. No tumours were observed in black mice in this study. In another study, a slightly increased incidence of lung adenomas was observed in female mice at the highest dose; however, there was a limited dose–response relationship and this tumour was common in the strain of mice used. In the absence of genotoxicity and on the basis of the weight of the evidence from the studies of carcinogenicity, the JMPR concluded that lindane is not likely to pose a carcinogenic risk to humans (FAO/WHO, 2002).

120. A 2-year rodent study by Thorpe and Walker, (1973), which was not cited by JMPR, reported an increase in the incidence of liver tumours in both males and female mice at a single tested dose of 400 ppm (equivalent to 60 mg/kg bw/day). COT considered that there is evidence that γ-HCH is carcinogenic in mice; however, its mechanism of action is associated to CAR which is not relevant to humans.

Observations in humans

121. As part of their review of organochlorine insecticides in 2004, the COC considered whether exposure to lindane was associated with an increased risk of breast cancer (COC, 2004). They concluded that:

“Lindane (γ-HCH) does not have any in vivo oestrogenic activity. It is not approved for use as a pesticide in the U.K. Exposure is likely to be negligible. The Committee have previously concluded that there is no biological rationale for including lindane in any epidemiology studies on risk of breast cancer. The Committee concluded there is no reason to undertake any further reviews of the association of this chemical with increased risk of breast cancer”.

122. Two investigations on the potential associations between γ-HCH body burden and breast cancer have been published since the COC statement but do not call into question its conclusions. A study by Mills and Yang (2006) reported no association after evaluation of a database covering a total of 23,513 women of Hispanic origin diagnosed with breast cancer in California during the years 1988-1999. Muir et al. (2004) conducted a spatial analysis in England, investigating associations between modelled ward-level pesticide application in 1991 (using data from the Pesticide Usage Survey from the Ministry of Agriculture Fisheries and Food) and cases of breast cancer in Lincolnshire and Leicestershire during the period 1989 to 1991, obtained from the Trent Cancer Registry. Findings were inconsistent, with a positive association in rural wards in Leicestershire but not Lincolnshire. Moreover, this was an ecological study, and as such, it was particularly prone to uncontrolled confounding. Also, it is unclear how closely agricultural pesticide application relates to exposure in the general population.

123. Three large case-control studies investigated associations between self-reported γ-HCH exposure and non-Hodgkin lymphoma (NHL). Blair et al (1998) combined information on 987 cases and 2895 population-based controls from three US cohorts, with questionnaire information about agricultural use of pesticides. There was an increased risk with reported use of lindane (OR 1.5, 95% CI 1.1-2.0) that remained statistically significant after adjustment for 10 of 17 other group or individual pesticide exposures. Lee et al (2004) subsequently analysed two of the same cohorts stratifying by farming and asthma status. In comparison with non-farmers who did not have asthma, farmers without asthma but with reported lindane exposure had an OR of 1.3 (95% CI 0.97-1.8) for NHL, and those with asthma and lindane exposure had an OR of 2.4 (1.0-5.7). A population-based Canadian study (McDuffie et al, 2001) of 517 cases and 1506 controls found an increased risk with exposure to lindane (OR 2.06, 1.01-4.22, based on 15 exposed cases). A further study from Iceland, where dipping of sheep with lindane was legally compulsory (Rafnsson et al, 2006) found a statistically significant association between sheep dipping and NHL in farmers. This finding was based on 45 cases and 221 controls nested in a cohort of 7882 sheep owners. The epidemiological studies described are compatible with a small effect of lindane on NHL, but because of important limitations in the assessment of exposures and control for confounders, the evidence is fairly weak.

124. A Californian case-control study found an exposure-response relationship between risk of prostate cancer risk and quartiles of an ecological measure of γ-HCH usage. The study included 222 cases and 1110 controls from a large cohort of members of a Farm Workers Union (Mills and Yang, 2003), and exposure was assessed as the pounds of pesticide active ingredient applied at county-level, as recorded by the California Department of Pesticide Regulation, in the places where the subjects had been employed. Strengths of this investigation were its large size, use of registry records, and assessment of exposure independently of, and prior to, diagnosis. Also, analyses adjusted for multiple other pesticide exposures. Weaknesses were the lack of individual -level exposure information and the possibility that relevant exposures could also have occurred before becoming a Union member or after leaving the Union.

125. A small number of studies have investigated associations of γ-HCH with Parkinson's disease, but their findings are inconclusive. Levels of γ-HCH were assessed in post-mortem brain tissue from 10 people with Parkinson's disease in a small UK study (Fleming et al, 1994). Levels of lindane in the substantia nigra (the brain tissue affected in Parkinson's disease) were significantly higher than in six neurologically normal controls, six with Alzheimer's disease and six with cortical Lewy body dementia. A US study (Corrigan et al , 2000) using frontal and/or occipital cortex did not detect lindane in 20 Parkinson's disease cases nor in 21 controls.

Allergy

126. No studies have been found associating α-HCH and incidence of allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

127. Table 10 summarises the ADI, TDI and RfDs that have been established or proposed for γ-HCH.

Table 10. ADI, TDIs and RfDs proposed for γ-HCH isomers.

Source of HBGV	HBGV µg/kg bw/day	Critical effect and species NOAEL/LOAEL mg/kg bw/day	UF	Study selected to derive HBGV
Integral Consulting (2011c)	RfD 0.01	Immunotoxicity in female rats LOAEL 0.012	1,000	Meera et al., 1992
RIVM (2001)	TDI 0.04	Immunotoxicity in female rats LOAEL 0.012	300	Meera et al., 1992
Health Canada (1992), cited in EFSA (2005)	Group TDI 0.3	Unknown		Not identified
JMPR (FAO/WHO, 2002)	ADI 5	Hepatotoxicity in rats NOAEL 0.47	100	Amyes et al., 1990

128. The RfD for γ-HCH proposed by Integral Consulting (2011c) was based on the study by Meera et al. (1992) on immunotoxicity in female rats exposed for 24 weeks with a LOAEL of 12 µg/kg bw/day (paragraph 113). An UF of 1,000 was applied (100 for inter- and intra-species variation, and 10 for extrapolation from LOAEL to NOAEL) resulting in a proposed RfD of 0.01 µg/kg bw per day.

129. The TDI for γ-HCH established by RIVM in 2001 was also based on the study by Meera et al. (1992). However they applied a total UF of 300 was applied (10 each for intra- and inter-species variability and 3 to compensate for the use of a LOAEL rather than a NOAEL), resulting in a TDI of 0.04 µg/kg bw per day. This study had criticised by JMPR in relation to the purity of the preparation used (~ 97%). However RIVM concluded that the JMPR argument was invalid since there were no indications at the time for impurities that would cause such a substantial higher toxicity of the substance tested.

130. JMPR established an ADI of 5 µg/kg bw on the basis of the NOAEL for hepatotoxicity, equivalent to 0.47 mg/kg bw/day from a long-term study of toxicity and carcinogenicity in rats (Amyes et al., 1990) (paragraph 110), applying a safety factor of 100. The Meeting established an acute RfD of 0.06 mg/kg bw on the basis of the NOAEL of 6 mg/kg bw in the study of acute neurotoxicity in rats (paragraph 107) in which clinical signs of toxicity (increased fore-limb grip strength and decreased grooming behaviour) were observed at higher doses, and a safety factor of 100. (FAO/WHO, 2002).

131. In 1992 Health Canada set a group TDI for all HCH isomers of 0.3 µg/kg bw (Feeley, 2005, personal communication to EFSA, 2005). Details on the derivation of this group TDI are not available in the public domain.

132. The COT noted that the toxicological database for γ-HCH (lindane) is much more extensive than for the other HCH isomers, and therefore establishing an HBGV was considered appropriate. The recent reproductive study extended two-generation reproduction study by Matsuura et al. (2005) provided a similar LOAEL (0.6 mg/kg

bw per day) to the study used by the JMPR to establish its ADI. However the COT could not discount the relevance of the immunotoxicity data of Meera et al. (1992) identifying a LOAEL of 12 µg/kg bw/day for a sensitive immunological marker. Taking into account that this is a sensitive endpoint, a UF of 3 for extrapolation from LOAEL to NOAEL is consistent with the COT approach (COT, 2004) together with a UF 100 for intra-/inter-species differences. Therefore the COT agreed with the RIVM TDI of 0.04 µg/kg bw per day. This should be considered a TDI rather than an ADI because γ-HCH now occurs as an environmental contaminant rather than as an approved pesticide.

Sources of γ-HCH and occurrence levels

Drinking water

133. In 2011, 3565 samples of treated water were analysed for γ-HCH in the UK. Four samples were reported to contain detectable concentrations (typical limit of detection is 0,003 µg/L). None exceeded the regulatory limit of 0.1µg/L (Drinking Water Inspectorate, personal communication, 2013).

Breast milk

134. A time-related decline in the levels of γ-HCH in breast milk is apparent from the scientific literature. Table 11 shows the concentrations of HCH isomers in breast milk from studies in UK populations published since 1982.

Table 11. UK studies measuring γ-HCH in breast milk published since 1982.

N	µg/kg milk fat					Mean µg/kg whole milk	% samples with detectable residues	Years of sample collection	Reference
	A. mean	G. mean	Med.	Min.	Max.				
102	30	N.D.	10	<10	270	1	55	1979-1980	Collins et al, 1982
-	-	-	-	-	-	<1	0	1984	MAFF, 1998
193	<20	N.D.	<20	<20	160	2	18	1989-1991	Dwarka et al, 1995
156	35 A or G?	35 A or G?	25	<8	200	<1	2	1997-1998	Harris et al, 1999
54	N.D.	0.8	0.6	N.D.	7.7	N.D.	91	2001-2003	Kalantzi et al, 2004

A. mean. Arithmetic mean, G. mean. Geometric mean, Med. Median, Min. Minimum, Max. Maximum, N.D. No data.

135. γ-HCH was not detected 92 samples of breastmilk from 48 donors in the UK sampled in 2001-2002, at a limit of detection of 10 µg/kg fat (Wooldridge et al, 2004).

136. In the context of the 3rd WHO human milk field study γ-HCH was analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine) and 11

pools from 6 non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA) (Malisch et al., 2004). In the pools from European countries the concentrations of γ-HCH ranged from < 1 to 13 µg/kg fat.

137. The occurrence levels of HCH isomers in breast milk in studies on populations in the United States and Europe published since 1995 are shown in Table 12.

Table 12. European studies measuring γ-HCH in breast milk published since 1995 in chronological order of sample collection.

Country (City/Region)	µg/kg milk fat median/mean/level as indicated (high percentile as indicated) (time data provided)		Years samples collection	Reference
Sweden	75 mean **(not indicated)** (1978)		1975-1990	Vaz, 1995
	27 mean **(not indicated)** (1990)			
Spain (Huelva / Andalucia)	80 mean **(highest sample, 200)** (after 1 month)		1989-1990	Martinez Montero, 1993
	71 mean **(highest sample, 130)** (after 3 months)			
Germany (Saxony)	5 median **(95%, 3,240)**		1992-1993	Raum et al, 1998
Germany (Saxony)	12 median **(not indicated)**		1992-1993	Schlaud et al, 1995
German (Saxony – Rural areas)	16 median **(not indicated)**		1992-1993	Schlaud et al, 1995
Greece (South West)	58 mean In whole milk **(not indicated)**		1995-1997	Schinas et al, 2000
Norway (Oslo)	0.7 mean **(not indicated)**		2000-2001	Polder et al, 2008
Norway (Tromsø)	0.3 mean **(not indicated)**		2000-2001	Polder et al, 2008
Germany (North Ryne-Westphalia)	20 levels **(not indicated)**		1984	P Fürst, personal communication to EFSA, 2005.
	< 1 mean **(not indicated)**		2001	
Spain (Almeria, agricultural area and Granada, urban area / Andalucia)	(1-7 days)	Almeria 0.31 mean **(not indicated)**	Not mentioned	Campoy et al, 2001
		Granada 1.60 mean **(not indicated)**		
	(6-12 days)	Almeria 0.28 mean **(not indicated)**		
		Granada 1.90 mean **(not indicated)**		
	(13-35 days)	Almeria 0.32 mean **(not indicated)**		
		Granada 0.82 mean **(not indicated)**		

Infant formula

138. Infant formulae are included in the UK national monitoring programme for pesticide residues in food, which is overseen by the Defra Expert Committee on Pesticide Residues in Food (PRiF). Infant formula was last surveyed in July-September 2009 (PRiF, 2010). γ-HCH was not detected at or above the reporting limits of 10 µg/kg each, i.e the current MRL.

139. Recent monitoring of the wider UK food supply, has not identified residues of lindane in whole milk at a reporting limit of 0.0004 mg/kg, indicates that levels in

cows' milk-based infant formulas are likely to be well below the MRL and reporting limit of 0.01 mg/kg. Soya milk was last included in the UK pesticide monitoring programme in 2006. Lindane was not detected at a reporting limit of 0.002 mg/kg; nor was it detected in soya pieces or tofu at this reporting limit. This indicates that levels in soy-based infant formula are likely to be well below 0.01 mg/kg.

140. A monitoring programme from 2001 to 2006 on marketed food including infant formula in Barcelona (Catalonia, Spain) did not find any infant formula sample at or above the quantification limits out of the 1484 samples analysed (Fontcuberta et al., 2008). The quantification limits in this study were 10 µg/kg in low fat food and 5 µg/kg in high fat food.

141. A study performed in Huelva (Andalucia, Spain) measured occurrence levels of γ-HCH and total HCH (sum of α-, β- and γ-HCH) in milk formula reconstituted as per manufacturers' instructions. The mean levels were 21 and 22 µg/kg respectively (Martínez Montero et al., 1993). No information was provided on range, median or percentiles, or on the levels of HCH in the water used for reconstitution.

142. A survey of the pesticide content of 25 infant formulae marketed in New Zealand was undertaken in 1996. It included a representative mixture of imported and New Zealand manufactured infant foods. Approximately 140 pesticides including γ-HCH were screened. γ-HCH was not detected with a limit of detection of 0.2 µg/kg (Cressey and Vannoort, 2003).

Weaning diet

143. Cereal-based, fruit and vegetables, and other products containing egg, fish, meat or cheese to be consumed by infants were last surveyed by PRiF in March (2011) (PRiF, 2011), August (2011) (PRiF, 2012) and February (2009) (PRiF, 2009) respectively. No γ-HCH was detected at or above the reporting limits of 10 µg/kg each.

144. Recent monitoring by PRiF of the wider UK food supply has not identified residues of lindane in any food commodities, consistent with it no longer being used as a pesticide and having a relatively short half-life of elimination in animals. For example, in the 2012 UK pesticide monitoring programme, lindane was not identified in any of 3657 samples of a wide range of agricultural commodities, processed foods and drinks (3537 samples, excluding infant foods). Reporting limits ranged from 0.0004 mg/kg for whole milk and lamb to 0.05 mg/kg for edible seeds; the reporting limit was 0.01 mg/kg for most commodities, including fruits, vegetables, cereals, butter, cheese and olives. It therefore appears very unlikely that lindane would be present in composite food products such as infant foods at levels close to the MRL and reporting limit of 0.01 mg/kg.

145. The Food Safety Authority of Ireland published in 2004 a report on surveillance of infant food for pesticide residues (Food Safety Authority of Ireland, 2004). Of the 41 infant food samples tested for 366 pesticide compounds including γ-HCH, representing 15006 individual results, there were no positive results for the presence of γ-HCH. The limit of quantification was 1 µg/kg. The samples were biscuits for infants (2 samples), infant formula (6 samples), fruit-based infant food (11

samples), vegetable/meat infant food (12 samples), cereal-based infant food (6 samples) and juices for infant and young children (3 samples).

146. A survey of the pesticide content of 30 weaning food products available in New Zealand was undertaken in 1996. It included a representative mixture of imported and New Zealand manufactured infant foods. Approximately 140 pesticides including γ-HCH were screened. γ-HCH was not detected with a limit of detection of 0.2 µg/kg (Cressey and Vannoort, 2003).

Exposure

147. An on-going market basket study performed between 1994 and 2003 in the Czech Republic, where HCHs were produced and used for a long time, indicated a decline of daily dietary intakes. The median daily intake for β-HCH in 1994 was 19.0 ng/kg bw (Ruprich et al., 1995) and in 2002 was 6.4 ng/kg bw (Ruprich et al., 2003).
148. Biomonitoring data in Germany indicate a decrease in exposure to γ-HCH. The third German Environmental Survey conducted in 1998 (GerES III) in blood samples from 4800 subjects geographically representative for the German population, age (18 to 69 years) and gender (Becker et al, 2002). The arithmetic mean and the maximum value for all subjects was <0.1 and 4.7 µg/L respectively. The limit of quantification (0.1 µg/L) was exceeded by 5.2% of the subjects. The subsequent survey (GerES IV) carried out between 2003-2006 in blood samples from 1063 children aged 7 to 14 from 150 randomly selected locations in Germany reported no subjects above the level of quantification of 0.076 µg/L (Schulz et al, 2009).

149. The values for consumption and body weight used for the estimation of UK infant exposure to γ-HCH are as described for α-HCH in paragraph 43.

Breast milk

150. The geometric mean value from the most recent UK study presented in Table 10, i.e. 0.8 µg/kg for γ-HCH (Kalantzi et al., 2004) was selected for the exposure estimation, calculated on the assumption that the fat content of breast milk was 3.5 % (see Table 13). The mean, rather than the maximum value was selected because the distribution of the data and comparison with other studies indicated that the maximum value might not be reliable, and also because levels would have been expected to decrease since the milk was sampled by Kalantzi et al. (2001-2003). Due to the reported decreases in γ-HCH in breast milk over time, these could possibly overestimate current exposure.

Table 13. Estimated exposure of infants to γ-HCH for average and high consumption of breast milk.

	Exposure (µg/kg bw day)			
Consumption	**0 – 4.0 months**	**>4.0 – 6.0 months**	**>6.0 – 9.0 months**	**>9.0 – 12.0 months**
Average	0.004	0.003	0.003	0.002
High level	0.006	0.004	0.004	0.004

Infant formula

151. As for α- and β-HCH, ½ MRL was selected to estimate the exposure of infants to γ-HCH, resulting in the same exposure of infants for α-and β- applies to γ-HCH (paragraph 46). DWI reported that the limit of detection for γ-HCH, 0.003 µg/L, was exceeded in 4 out of 3565 analyses. If γ-HCH isomer was present at 0.003 µg/L in water used to reconstitute infant formula, the exposure from the water would be 0.004µg, equivalent to 0.0006 µg/kg bw day for infants younger than 4 months with an average weight of 5.9 kg. This is likely to have a negligible impact on the total from reconstituted formula.

Weaning diet

152. The maximum exposure estimates calculated from solid infant foods for α- and β- also apply to γ-HCH given the selection of ½ MRL common to all isomers (paragraph 47).

Risk characterisation

153. The estimated maximum exposures of infants to γ-HCH are presented in Table 14. Estimated exposure of breast fed infants is below the TDI of 0.04 µg/kg bw per day and not a concern.

154. The estimated exposure from infant formula exceeds the TDI for both average and high consumers in all age groups. The available data indicate that exposure from water used to reconstitute infant formula would be negligible. Similarly, the estimated exposure to γ-HCH from solid infant food for average and high consumers exceeds the TDI set up by COT. However since these estimates are based on non-quantified data it is possible that actual exposures are lower since exposure estimates are based on non-quantified data and the occurrence levels in ingredients used to reconstitute infant formula and infant food products are substantially below ½ MRL. .

155. The estimated exposures for all age groups and consumers from breast milk, infant formula and infant food are substantially below the ARfD 60 µg/kg bw/day established by JMPR.

Table 14. Potential maximum/estimated exposure of infants to γ-HCH from consumption of breast milk, infant formula and infant foods

Isomer	Consumption	Exposure (µg/kg bw/day)			
		0 – 3 months	4 – 6 months	7 – 9 months	10 – 12 months
Breast milk	Average	0.003	0.003	0.002	0.002
	High level	0.006	0.004	0.004	0.004
Infant formula	Average	< 0.09	< 0.07	< 0.06	< 0.06

(assuming compliance with EU legislation)	High level	< 0.14	< 0.10	< 0.09	< 0.08
Infant food (assuming compliance with EU legislation)	Average	N/A	N/A	< 0.18	
	High level	N/A	N/A	< 0.27	

Overall conclusions

156. α-, β- and γ-HCH are well absorbed and distributed throughout the body. They are capable of inducing drug metabolism enzymes and their major metabolic pathway is through dechlorination. Their excretion profile varies depending on the isomer.

157. The COT concluded that α, β and γ-HCH are not importantly genotoxic. There is some evidence for carcinogenicity in rodents, but this is unlikely to be relevant to humans.

158. Currently, pesticide use of HCH isomers is banned. Occurrence and exposure levels in the scientific literature demonstrate a trend of reduction world-wide.

159. Food items are periodically surveyed for quantification of occurrence levels of pesticides with only those levels exceeding the reporting limit (which has the same value as the MRL) being recorded. The occurrence levels for HCH isomers for infant formula and infant food are all below the MRL. In view of the lack of quantified occurrence levels, ½ MRL for infant food was selected as pragmatic level to estimate exposures to infant formula and food. Using ½ MRL is still likely to overestimate the occurrence levels of HCH isomers in infant formula and food since measured levels in fatty food (eggs, butter, whole milk, lamb), which would be expected to contain the highest levels and have lower MRLs than infant food were substantially below ½ MRL. These ingredients are commonly used in infant formula and infant products.

160. The COT concluded for α-HCH that a MOE greater than 1,000 compared to the NOAEL of 0.1 mg/kg bw/day for liver hypertrophy in rats would not be of health concern. This value of 1,000 was based on default UFs for intra-/inter-species differences and an additional factor of 10 for the gaps in the database. Estimated exposure for breastfeeding infants and infants consuming formula resulted in MOEs greater than 1,000 and would not be a health concern. The MOE from estimated exposures for infants consuming infant food would be 600 or 400 for average and high level consumers. However, since based on levels in fatty food, the assumption of using ½ MRL of infant food for estimating the exposure from infant food would overestimate exposure and considering the data overall, there is unlikely to be a health concern for infant food.

161. The COT concluded for β-HCH that a MOE greater than 3,000 compared to the LOAEL of 0.18 mg/kg bw/day for centrilobular hypertrophy in rats would not be of health concern. The MOE was based on default UFs for intra-/inter-species differences, and an additional factor of 3 for extrapolation from a LOAEL to a NOAEL and 10 for gaps in the database. Estimated exposures for breastfeeding infants and

infants consuming formula resulted in MOEs greater than 3000 for average consumers from the ages of 4 and 6 months upwards which would not be a health concern. Estimated exposures for younger and high level consumers of breast milk and infant formula resulted in MOEs below 3000, these MOEs increased as infants grew older. Estimated exposures from infant food resulted in MOEs below 3000. However, since based on levels in fatty food, the assumption of using ½ MRL of infant food for estimating the exposure from infant food would overestimate exposure and considering the data overall, there is unlikely to be a health concern for infant food.

162. COT established a TDI 0.04 µg/kg bw/day for γ-HCH on the basis of a LOAEL of 12 µg/kg bw/day for immunotoxic effects in female rats with UFs of 100 for intra-/inter-species differences and 3 for extrapolation from LOAEL to NOAEL. Estimated exposures of breastfeeding infants were below the TDI and were not a health concern. Estimated exposures of infants consuming formula were up to three fold above the TDI and decreased with age to levels slightly above it and contributions from formula would be expected to continue to decline as the infant grows older to levels below the TDI. In addition, based on levels in milk, the assumption of using ½ MRL would overestimate exposure. Estimated exposures of infants consuming infant foods were above the TDI. However, these exposures would be unlikely to be of health concern since based on levels in fatty food, the assumption of using ½ MRL of infant food for estimating the exposure from infant food would overestimate exposure, the trend of decreasing levels in the environment and that such exposures would only occur over a limited time period whilst the TDI is an intake expected to be without appreciable risk to health even if consumed over an entire life-time.

Secretariat
October 2013

ABBREVIATIONS

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body Mass Index
CAR	Constitutive androstane receptor
CERHR	Center for the Evaluation of Risks to Human Reproduction
CNS	Central Nervous System
COC	Committee on carcinogenicity of chemicals in food, consumer products and the environment
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
DNSIYC	Dietary and Nutrition Survey of Infants and Young Children
DWI	Drinking Water Inspectorate
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
GABA	Gamma-amino butyric acid
GerES	German Environmental Survey
GST-P	Glutathione S-transferase placental
HBGV	Health based guidance value
HCH	Hexachlorocyclohexane
IPCS	International Program on Chemical Safety
JMPR	WHO/FAO Joint Meeting on Pesticide Residues
LD50	Lethal dose 50
LLNA	Local lymph node assay
MAFF	Ministry of Agriculture, Fisheries and Food
MCF-7	Human mammary carcinoma cells
MOA	Mode of action
MOE	Margin of exposure
MRL	Maximum Residue Limit
NHL	Non-Hodgkins Lymphoma
LOAEL	Lowest observed adverse effect
MOE	Margin of Exposure
NOAEL	Non observed adverse effect
OPP	Office of Pesticides Programme
OR	Odd ratio
PRiF	Defra Expert Committee on Pesticide Residues in Food
PC-3	Human prostate cancer cells
RfD	Reference dose
RIVM	Rijksinsituut Voor Volksgezondheid En Milieu
RL	Reporting limit
SACN	Scientific Advisory Committee on Nutrition
SD	Standard Deviation
TDI	Tolerable Daily Intake
US EPA	United States Environmental Protection Agency

US FDA	United States Food and Drug Administration
UF	Uncertainty factor
WHO	World Health Organization

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Search strategy

Websites and databases interrogated which have been the sources of most bibliographic references are:

- JMPR
- IPCS-WHO
- EFSA
- Integral Consulting

Pubmed has been used in order to search for complementary information or gaps not covered by the mentioned reviews. The specific search terms are listed below:

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND breast milk
Search Dates (From/To) - From 1965 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND infant formula
Search Dates (From/To) - From 1970 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND infant food
Search Dates (From/To) - From 1970 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND weaning
Search Dates (From/To) - From 1970 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND epidemiology
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND genetic toxicology
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND occurrence levels
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND exposure
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND acute toxicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND repeat toxicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND neurotoxicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND immunotoxicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND reproductive toxicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND carcinogenicity rodent
Search Dates (From/To) – From 1992 to present

HCHs/α-HCH/β-HCH/γ-HCH/lindane AND half-life blood
Search Dates (From/To) – From 1992 to present

A number of the studies cited in the section on “Observations in humans” for the HCH isomers have been selected by reviewing the bibliography of studies captured with the listed search terms.

Other sources used are:

- Medical and Healthcare products Regulatory Agency
- Drinking Water Inspectorate
- COT
- COC
- FSA
- ATSDR
- USFDA
- USEPA