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

COT statement on the potential risks from 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 among other things, the review should include chemicals which in 2009 had been added to the annexes of the Stockholm Convention on Persistent Organic Pollutants[1].

2. This statement gives an overview of potential risks from occurrence in the infant diet of three such chemicals, α-, β- and γ-hexachlorocyclohexanes (HCHs). None of the Government's current dietary recommendations for infants and young children relates to HCHs.

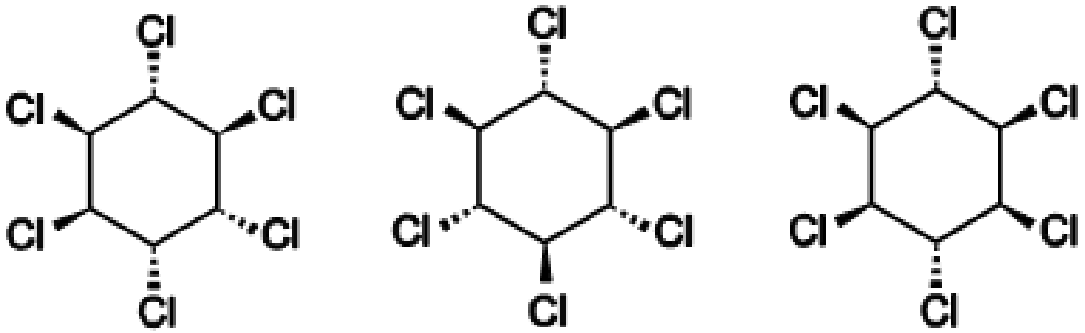
3. Lindane (γ-HCH) has been evaluated by the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) (WHO-IPCS, 1991) and by the Joint Food and Agriculture Organization (FAO)/WHO Meeting on Pesticide Residues (JMPR) (FAO/WHO, 2002). In addition, α- and β-HCH have been 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) have published reviews of the toxicity of HCH isomers (USEPA, 2001; ATSDR, 2005). On behalf of Syngenta Crop Protection and Stauffer Management Company, Integral Consulting recently reviewed the toxicity of α-, β- and γ-HCH, using literature published up to March

[1] http://chm.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx

2011 (Integral Consulting, 2011a, b, c). These reviews were used as the primary sources of information in this statement. Literature searches, as described in the appended search strategy, were conducted through to October 2012 to identify any further relevant publications. Additional papers identified by COT members were also considered.

General background on HCHs

4. There are eight HCH isomers; α-, β-, γ-, δ-, ε-, ζ-, η- and θ-HCH. This review focuses on α- β- and γ-HCH, which are listed for elimination in Annex A of the Stockholm convention. Their structures are shown in Figure 1.



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

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

5. Due to their lipophilic properties and persistence in the environment, β-HCH, and to a lesser extent, α-HCH and γ-HCH, bioaccumulate and biomagnify in the food chain. They are distributed globally, with transfer from warmer to colder regions through evaporation and condensation (EFSA, 2005).

6. HCHs have been used as pesticides. The term “lindane” has been commonly used for HCH mixtures used as pesticides in which γ-HCH was >99% of total HCH, and throughout this statement the term is used only with that meaning (elsewhere “lindane”, has sometimes also been used as a synonym for γ-HCH).

7. Pesticidal use of products in which γ-HCH made up less than 99.0 % of all HCHs was banned in the EU by Council Directive 79/117/EEC of 21 December 1978. Authorisation for use of lindane as a pesticide in the EU was withdrawn by Commission Decision 2000/801/EC of 20 December 2000. This action was taken primarily because of concerns about its safety for operators, the fate and behaviour of γ-HCH in the environment and effects on non-target organisms.

8. Lindane has been used for topical insecticide treatment in humans and animals. 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 containing lindane are

approved by the US Food and Drug Agency for medical purposes (USFDA, 2007). Lindane is typically used at a concentration of 1 % (Reynolds, 1996).

9. The maximum levels of pesticides that are currently permitted in the EU in foods sold for infants are set out 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 permitted levels in other food products are 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 set specifically for a product or product type. This default applies to HCHs in infant and follow-on formula and in processed cereal foods and baby foods for infants and young children. As regards other food products for human consumption, several different MRLs apply depending on the HCH isomer and the food commodity in question, although for most, the default MRL applies. MRLs range from 0.004 to 0.2 mg/kg for α-HCH, from 0.003 to 0.1 mg/kg for β-HCH, and from 0.001 to 1 mg/kg for γ-HCH (Directive 2013/212/EC).

γ-HCH

10. The gamma isomer (γ-HCH) is considered first in this statement, because more extensive toxicological data are available for the compound, and there is some scope for extrapolation to the alpha and beta isomers

Toxicokinetics

11. γ-HCH is extensively absorbed in mice and rats after oral dosing. Absorption is rapid, and after single administration of 20 mg/kg body weight (bw), levels in blood peaked at 40 minutes to 5 hours, and then plateaued (FAO/WHO, 2002). Absorption through skin has been demonstrated in human volunteers when the compound was applied in solvents such as white spirit and alcohol (Dick et al., 1997).

12. γ-HCH is widely distributed throughout the body in rodents. In mice and rats, results from several studies showed similar patterns of distribution after dietary administration of lindane, with radiolabel detected mostly in fat, followed by brain, kidney, muscle, liver and ovary. (FAO/WHO, 2002). Based on studies in workers, the mean half-life for elimination from plasma has been reported to be 8 to 10 days in humans (Health Council, 2001).

13. The metabolism of γ-HCH in mammals is extensive, involving dehydrogenation, dechlorination, hydroxylation and dehydrochlorination, which may then be followed by conjugation. Cytochrome P450 (CYP) enzymes appear to be involved in the phase I metabolism. The predominant phase 1 metabolite is 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, pentachlorocyclohexene, and conjugates with glutathione, glucuronic acid and sulphate.

14. The urine is the major route by which metabolites of γ-HCH are excreted, a smaller proportion being eliminated in the faeces. The elimination 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).

Toxicity of γ-HCH

15. Oral LD50 values for γ-HCH have been reported as 56 to 250 mg/kg bw in mice and 140 to 190 mg/kg bw in rats (FAO/WHO, 2002).

16. Short term administration of γ-HCH to rats has been shown to induce CYPs. For example, Parmar et al. (2003) reported a dose-dependent induction of CYP 1A1/1A2, 2B1/2B2 and 2E1 enzymes following oral administration of γ-HCH to rats at 2.5 to 15 mg/kg bw/day for dosing periods of 5 to 21 days.

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

18. 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 (Amyes, 1990), which was used by JMPR (FAO/WHO, 2002) as the basis for its Acceptable Daily Intake (ADI), lindane (purity 99.75%) was fed to rats at dietary concentrations of 1, 10, 100 or 400 ppm (estimated by the authors to correspond to to 0.05, 0.47, 4.8 and 20 mg/kg bw/day for males, and to 0.06, 0.59, 6.0 and 24 mg/kg bw/day for females. Males and females at the highest dose had increased absolute and relative liver weights at all interim sacrifices (weeks 4, 26, 52, 104), although statistical significance was not always reached. At the end of the study, the absolute and relative liver weights were significantly increased at the top dose, by 21% and 38% respectively in males, and by 32% and 34% respectively in females. At 100 ppm, the absolute liver weights were 8.6-11% higher than control (not statistically significant) and the relative liver weights were 14-18% higher than control (statistically significant). Significant increases in absolute and relative spleen weights were also seen at week 52 and in relative spleen weights at week 104. The incidence of periacinar hepatocytic hypertrophy was significantly increased at both 100 ppm and 400 ppm. JMPR calculated that survival was reduced in both males and females at 100 ppm and 400 ppm, and concluded that the NOAEL was 10 ppm in the diet, corresponding to 0.47 mg/kg bw per day, on the basis of increased spleen weights, toxic effects on the liver, and reduced survival at 100 ppm (corresponding to 4.7 mg/kg bw per day) (FAO/WHO, 2002).

19. Many studies on the neurotoxicity of γ-HCH were 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, with dietary administration of γ-HCH from gestational day 6 to postnatal day 10, the NOAEL for maternal toxicity was 4.2 mg/kg bw per day on the basis of decreased body weight, decreased food consumption and increased reactivity to handling.

20. Meera et al. (1992) investigated a number of different functional immunological endpoints in female mice exposed via the diet to 0.012, 0.12 and 1.2 mg/kg bw/day of an HCH preparation containing 97% γ-HCH, for 4, 8, 12, 16, 20 and 24 weeks. At the lowest dose, the lymphocyte proliferation response to concanavalin A was significantly increased until week 8, after which it decreased to below the level in controls. With the other doses, it was statistically significantly higher than in controls at week 4 and decreased over time. The lymphoproliferative response to lipopolysaccharide in mice was statistically significantly above control levels in week 4 at all doses, and again decreased with time. This was paralleled by an increase in plaque-forming cells at all doses at 4 weeks and a subsequent reduction. Necrosis of thymus, lymph nodes and spleen was reported at 1.2 mg/kg bw/day.

21. In a study by Wing et al. (2000), which is described in FAO/WHO (2002), immunotoxicity was investigated in mice fed diets containing lindane (purity, 99.78%) at 10, 40 or 160 ppm for 39 weeks. Blood was collected from the retro-orbital sinus and the lymphocyte populations were identified with antibodies to CD3, CD4 and CD8 for T lymphocytes, CD19 for B lymphocytes and DX5 for natural killer (NK) cells. A statistically significant ($p < 0.05$) 55% increase in NK cells was reported in females given lindane at 160 ppm (32 mg/kg bw). This was the only lymphocyte parameter affected. JMPR (FAO/WHO, 2002) concluded that under the conditions of this study, lindane did not affect the number or proportion of circulating lymphocytes.

22. Endocrine outcomes have been investigated in a number of studies. JMPR noted that lindane had shown anti-estrogenic effects in several studies at doses of 5 mg/kg bw/day or more (FAO/WHO, 2002). In male rats, dermal exposure to γ-HCH at 50 or 100 mg/kg bw/day led to a decrease in serum testosterone levels, epididymal sperm count and 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 following dosing during pregnancy at 15 and 30 mg/kg bw/day (La Sala et al., 2009). This was supported by the observation that γ-HCH increased the rate of apoptosis in mouse primordial germ cells in vitro (La Sala et al., 2009).

23. A large number of studies have investigated the reproductive toxicity of γ-HCH (ATSDR, 2005). The outcomes investigated have been diverse and in some cases results have been inconsistent between studies. In rats, effects such as delayed vaginal opening, decreased ovarian weight and decreased number of fetuses have been reported. In the offspring, the effects observed have been decreased weight and viability of pups and increased spleen weight. In a multigeneration study in mink, γ-HCH at 1 mg/kg bw/day from 3 or 6 weeks premating to 8 or 10 weeks postpartum induced effects such as reduced mating receptivity, increased embryo loss and reduced whelping rate (in the F1 generation) reduced litter size (in the F2 generation), and reduced testicular size (in the F3 generation).

24. In an extended two-generation reproductive study by Matsuura et al. (2005), rats were fed γ-HCH (purity 99.5%) at 10, 60 or 300 ppm in the diet from 10 weeks before mating until terminal necropsy in the males; and through mating, gestation and lactation until F1 weaning at post-natal day 21 in females. After weaning, the F1 generation were treated in the same manner as the F0 up to termination at 21-23 weeks of age. General toxicity was seen at 300 ppm in F0 and F1 adults, which included decreased body weight gain, reduced food consumption, and in females, deaths. In addition, convulsions and irritability were observed at 300 ppm in the F1 females during the perinatal period. Reproductive effects included lack of nursing and retrieval behaviour at 300 ppm, possibly due to effects on the nervous system, but there were no effects on mating, fertility, pregnancy or parturition. The F1 and F2 offspring (both sexes) of mothers exposed at 300 ppm had significantly lower body weight at post-natal days 0-21. No changes were found in endpoints for endocrine-disrupting activity (anogenital distance, nipple development, sexual maturation, oestrous cycle, spermatogenesis, sex organ weights or blood hormone concentrations), or in a series of behavioural tests conducted in the F1 generation. There were statistically significant increases in relative liver weight in F0 males at all doses but absolute liver weight was increased only at the top dose. In F0 females relative and absolute liver weights were increased in the mid and high dose groups but not the low dose group. In F1 males there was an increase in relative liver weight at the top dose, and in F1 females at the mid and high doses. The activities of a number of hepatic drug-metabolising enzymes were measured in the F0 and F1 animals, and were increased at 60 at 300 ppm in both sexes and generations, the results suggesting that lindane most strongly induces CYP2B enzymes, but also upregulates a number of other drug-metabolising enzymes, such as CYP1A, CYP3A and UDP-glucuronosyltransferase. Histopathogical examinations of the parental generations revealed a statistically significant increase in centrilobular hepatocyte hypertrophy in both sexes at 60 and 300 ppm. The authors ascribed the centrilobular hepatocyte hypertrophy to an adaptive response following induction of drug-metabolising enzymes. Based on the decreased weight gain in the offspring, the NOAEL in this study was at the lowest dietary concentration of 10 ppm, calculated by the authors to correspond to a maternal dose of 0.6 mg/kg bw/day.

Genotoxicity

25. JMPR reviewed a large number of studies that explored the genotoxicity of lindane, including in vitro assays for bacterial and mammalian cell mutation, and DNA repair, and assays in vivo for chromosomal aberrations, sister chromatid exchanges and dominant lethal mutations. They concluded that genotoxicity was found only at high concentrations of lindane resulting in cytotoxicity or precipitation of the compound, and that lindane is not genotoxic (FAO/WHO, 2002).

26. Since the JMPR evaluation, Kalantzi et al. (2004a) have reported a weakly positive result for 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 was reported in MCF-7 and human prostate cancer (PC-3) cell lines at concentrations in the region of 1-100 pM (testing of higher concentrations was not

reported). COT concluded that the overall balance of evidence indicated that γ-HCH is not mutagenic.

Carcinogenicity

27. 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 on the role of genetic background in the latency and frequency 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 (21 mg/kg bw/day); however, JMPR noted this tumour was common in the strain of mice used. In the absence of genotoxicity and on 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).

28. A 2-year mouse dietary study by Thorpe and Walker (1973), which was not cited by JMPR, reported an increase in liver enlargement, hyperplastic foci and parenchymal cell tumours in both males and females at a single tested dose level of 400 ppm γ-HCH (equivalent to 60 mg/kg bw/day). Liver tumours were also observed in mice fed 500 ppm phenobarbitone. The hepatic effects of γ-HCH, including increased liver weight, centrilobular hypertrophy and induction of CYP2B enzymes, were similar to those produced by phenobarbitone (Elcombe et al., 2014). Phenobarbitone is generally accepted to be a non-genotoxic carcinogen with a mode of action (MOA) involving activation of the constitutive androstane receptor (CAR), which results in increased cell proliferation, liver hypertrophy, induction of CYP2B enzymes, formation of altered hepatic foci, and ultimately in liver tumours. Elcombe et al. (2014) concluded that species differences between rodents and humans mean that this MOA is not qualitatively plausible for humans. This conclusion was supported by data from a number of epidemiological studies conducted in human populations with chronic exposure to phenobarbitone, in which there was no clear evidence for increased liver tumour risk (Elcombe et al., 2014). The Committee considered that γ-HCH is carcinogenic in mice through a non-genotoxic mechanism involving CAR activation and unlikely to be a human liver carcinogen.

Observations in humans

29. In 2004, as part of a review of organochlorine insecticides, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (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".

30. Two investigations on the potential association between γ-HCH body burden and breast cancer have been published since the COC statement but do not call into question its conclusions. These investigations were carried out after the ban on lindane in the EU in 2000. 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 who were diagnosed with breast cancer in California during the years 1988-1999. Muir et al. (2004) conducted a spatial analysis in England, investigating associations between ward-level pesticide application in 1991 (when use of lindane use was still authorised), modelled using data from the Pesticide Usage Survey of the Ministry of Agriculture, Fisheries and Food (MAFF), and the incidence of breast cancer in Lincolnshire and Leicestershire during 1989 to 1991 (from data provided by the Trent Cancer Registry). Findings were inconsistent, with a positive association in rural wards in Leicestershire but not Lincolnshire. This was an ecological study, and as such, it was particularly prone to uncontrolled confounding. Also, it is unclear how closely local agricultural pesticide application relates to exposures in the general population.

31. Three large case-control studies investigated associations between self-reported exposure to γ-HCH and non-Hodgkin lymphoma (NHL). Blair et al. (1998) analysed questionnaire information regarding agricultural use of pesticides by 987 cases and 2895 population-based controls, who were white men living in Kansas, Nebraska, Iowa and Minnesota. Risk of NHL was increased in those who reported use of lindane (odds ratio (OR) 1.5, 95% CI 1.1-2.0), and this association remained statistically significant after adjustment for exposure to 10 other pesticides or combinations of pesticides. 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, 2006), found a statistically significant association between sheep dipping and NHL in farmers. This 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.

32. A Californian case-control study found a positive exposure-response relationship between risk of prostate cancer 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 direct individual-level information on exposure and the

possibility that relevant exposures could also have occurred before becoming a Union member or after leaving the Union.

33. 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 γ-HCH 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) did not detect γ-HCH in the frontal and/or occipital cortex of 20 cases of Parkinson's disease, nor in 21 controls.

34. The association between exposure to lindane and endometriosis has been investigated in two studies, with differing outcomes. A case-control study by Upson et al. (2013), nested within a population enrolled in an integrated health care system in Washington State (USA), included 248 surgically confirmed cases and 538 controls. It found no associations between lindane and endometriosis, but levels of γ-HCH were below the limit of detection (LOD) in 53.9% of individuals. A study by Buck Louis et al. (2012) investigated an operative group of 473 women aged 18-44 years who underwent laparoscopy or laparotomy at 14 participating clinical centres across the USA during 2007 to 2009 and a population-based sample of 127 women matched on age and residence. Diagnosis was made by visualisation and histologic confirmation in the operative group and by Magnetic Resonance Imaging (MRI) in the population sample. Concentrations of γ-HCH were measured in both omental fat and serum in the operative group and were higher in fat than in serum for several persistent organic pollutants. In the operative group, endometriosis was significantly associated with γ-HCH in fat (adjusted OR (AOR) per 1 standard deviation (SD) increase in log-transformed γ-HCH = 1.27; 95% CI: 1.01, 1.59), but not with γ-HCH in serum (AOR per 1 SD increase in log-transformed γ-HCH = 0.81; 95% CI 0.56, 1.18). However, γ-HCH in serum was significantly associated with endometriosis in the population sample (AOR 1.87; 95% CI: 1.04, 3.36).

35. An in-vitro investigation exposing human sperm to various concentrations of γ-HCH showed no disruptive effects on sperm function (Pflieger-Bruss et al., 2006). In a study comparing HCH in fat samples from 18 children with undescended testes who were undergoing surgery, and 30 surgical controls (Hosie et al. 2000), no association was found.

Allergy

36. No studies have been found that relate γ-HCH to the occurrence of allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

37. Table 1 summarises ADI, tolerable daily intake (TDI) and reference dose (RfD) values that have been proposed for γ-HCH.

Table 1. ADIs, TDIs and RfD proposed for γ-HCH.

Source of HBGV	HBGV µg/kg bw/day	Critical effect and species NOAEL/LOAEL in mg/kg bw/day	Uncertainty factor	Study selected to derive HBGV
Health Canada (1992), cited in EFSA (2005)	Group TDI 0.3 for all HCH isomers	Unknown		
RIVM (2001)	TDI 0.04	Immunotoxicity in female rats LOAEL 0.012	300	Meera et al., 1992
JMPR (FAO/WHO, 2002)	ADI 5	Decreased survival, liver and spleen effects in rats NOAEL 0.47	100	Amyes, 1990
Health Canada (2010)	ADI 0.5	Decreased survival, liver and spleen effects in rats NOAEL 0.47	1000	Amyes, 1990
Integral Consulting (2011c)	RfD 0.01	Immunotoxicity in female rats LOAEL 0.012	1,000	Meera et al., 1992

38. 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 of the derivation of this group TDI are not available in the public domain.

39. Lindane has undergone a number of evaluations by JMPR since 1963. In 1997 (FAO/WHO, 1997) JMPR established a temporary ADI of 1 µg/kg bw, derived from a NOAEL of 0.47 mg/kg bw/day on the basis of deaths and hepatic toxicity in a 2-year study of toxicity and carcinogenicity in rats by Amyes (1990) (paragraph 18). A safety factor of 500 was applied because immunotoxicity had been reported in studies of an HCH preparation containing 97% γ-HCH (i.e. Meera et al., 1992; see paragraph 20) or of unknown purity, and hence not meeting the specifications for authorised pesticidal uses of lindane at that time. The ADI was made temporary pending clarification of the immunotoxicity of lindane that met the specification (≥ 99% γ-HCH).

40. The TDI for γ-HCH established by the Dutch National Institute of Public Health and the Environment (RIVM) in 2001 was based on the immunotoxicity LOAEL of 12 µg/kg bw per day in the study by Meera et al. (1992). RIVM applied a total uncertainty factor of 300 (made up of factors of 10 each for intra- and inter-species variability and 3 to compensate for the use of a LOAEL rather than a NOAEL, taking into account the rather marginal toxic responses at the LOAEL), and this resulted in a TDI of 0.04 µg/kg bw.

41. In response to the 1997 JMPR conclusions, the immunotoxicity study of Wing et al. (2000) (paragraph 21) was submitted to JMPR for evaluation at its 2002 meeting (FAO/WHO, 2002). On the basis of a full re-evaluation of lindane, including the study of Wing et al. (2000), JMPR concluded that lindane is not immunotoxic and established an ADI of 5 µg/kg bw based on the NOAEL of 0.47 mg/kg bw/day from

the long-term study of toxicity and carcinogenicity in rats (Amyes, 1990) (paragraph 18), in which an increased incidence of periacinar hepatocellular hypertrophy, increased liver and spleen weights, and increased mortality occurred at higher doses. To this, they applied a safety factor of 100. JMPR also established an acute reference dose (ARfD) of 60 µg/kg bw based on the NOAEL of 6 mg/kg bw in a study of acute neurotoxicity in rats (paragraph 19), in which increased fore-limb grip strength and decreased grooming behaviour were observed at higher doses (FAO/WHO, 2002). This again incorporated a safety factor of 100.

42. Health Canada subsequently published a re-evaluation of lindane, which set an ADI of 0.5 µg/kg bw/day. This was based on the same data (Amyes, 1990) as the ADI set by JMPR, but incorporated an additional 10-fold factor as required under the Canadian Pest Control Products Act for substances with the potential for effects on unborn and developing children (Health Canada, 2009).

43. The RfD for γ-HCH proposed by Integral Consulting (2011c) was also based on the LOAEL of 12 µg/kg bw/day from the immunotoxicity study of Meera et al. (1992). An uncertainty factor of 1,000 was applied (100 for inter- and intra-species variation, and 10 for extrapolation from a LOAEL to a NOAEL), resulting in a proposed RfD of 0.01 µg/kg bw per day.

44. The COT concluded that the toxicological database for γ-HCH was sufficient to establish an HBGV. The Committee noted that the study by Meera et al. (1992) showed consistent immunomodulation across several assays of immune function, including the haemolytic plaque-forming cell assay, which is considered the most predictive of any single immune function test. In contrast, the Wing study drew conclusions based on a lack of change in the proportions of a limited number of circulating cell types in peripheral blood. Furthermore the methods and results of the Meera study, published in a peer-reviewed journal, are described in adequate detail for the conclusions to be evaluated. In contrast, the description of the Wing study in the JMPR monograph (FAO/WHO, 2002) is very limited, making it difficult to determine its quality. Therefore the COT agreed with the approach taken by the RIVM to derive a TDI of 0.04 µg/kg bw based on the LOAEL from the study by Meera et al. (1992), and used this TDI in its current evaluation.

Sources of γ-HCH and occurrence levels

Drinking water

45. In 2011, 3,565 samples of treated water were analysed for γ-HCH in the UK. Four samples were reported to contain detectable concentrations (a typical limit of detection was 0.003 µg/L). None exceeded the regulatory limit of 0.1µg/L (DWI, 2012).

Breast milk

46. Table 2 shows the concentrations of γ-HCH in breast milk from studies in UK populations published since 1982. Concentrations in breast milk sampled since the use of lindane was phased out in 2000 are clearly lower than in earlier samples.

Table 2. Concentrations of γ-HCH in breast milk sampled in the UK from reports 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.R.	10	<10	270	1	55	1979-1980	Collins et al., 1982
-	-	-	-	-	-	<1	0	1984	MAFF, 1998
193	<20	N.R.	<20	<20	160	<1	18	1989-1991	Dwarka et al., 1995
156	35[a]	N.R.	25	<8	200	<1	2	1997-1998	Harris et al., 1999
48	<10	<10	<10	<10	<10	<0.35[b]	0	2001-2002	Woolridge et al., 2004
54	N.R.	0.8	0.6	N.D.	7.7	0.028 [b]	91[c]	2001-2003	Kalantzi et al., 2004b

A. mean. Arithmetic mean, G. mean. Geometric mean, Med. Median, Min. Minimum, Max. Maximum, N.R. Not reported.
[a] Confirmed as arithmetic mean by C Harris (personal communication)
[b] Estimated assuming 3.5% fat in whole milk
[c] Limits of detection appear to have been much lower in this study than in earlier investigations (<0.001 mg/kg fat *cf.* 0.008-0.02 mg/kg fat)

47. As part of the 3rd WHO human milk field study, γ-HCH was analysed in 16 samples of pooled human milk 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 samples from European countries the concentrations of γ-HCH ranged from < 1 to 13 µg/kg fat.

48. Levels of HCH isomers in breast milk in studies on populations in other European countries published since 1995 are summarised in Table 3. As in the UK, levels have decreased since use of lindane was phased out.

Table 3. Concentrations of γ-HCH in breast milk sampled in other European countries from reports published since 1995.

Country (City/Region)	µg/kg milk fat		Years of sample collection	Reference
Sweden	Mean 75 in 1978		1975-1990	Vaz, 1995
	Mean 27 in 1990			
Spain (Huelva / Andalucia)	Mean 80; maximum 200 (after 1 month breastfeeding)		1989-1990	Martinez Montero et al., 1993
	Mean 71; maximum 130 (after 3 months breastfeeding)			
Germany (Saxony)	Median 5; 95th centile 3,240		1992-1993	Raum et al., 1998
Germany (Saxony)	Median 12		1992-1993	Schlaud et al., 1995
German (Saxony – Rural areas)	Median 16		1992-1993	Schlaud et al., 1995
Greece (South West)	Mean 58 (µg/L in whole milk)		1995-1997	Schinas et al., 2000
Norway (Oslo)	Mean 0.7		2000-2001	Polder et al., 2008
Norway (Tromsø)	Mean 0.3		2000-2001	Polder et al., 2008
Germany (North Rhine-Westphalia)	Mean 20		1984	P Fürst, personal communication to EFSA, 2005.
	Mean < 1		2001	
Spain (Almeria, agricultural area and Granada, urban area / Andalucia)	(1-7 days)	Mean 0.31 in Almeria	Not reported	Campoy et al., 2001
		Mean 1.60 in Granada		
	(6-12 days)	Mean 0.28 in Almeria		
		Mean 1.90 in Granada		
	(13-35 days)	Mean 0.32 in Almeria		
		Mean 0.82 in Granada		

Infant formula

49. Infant formulae are included in the UK national monitoring programme for pesticide residues in food, which is overseen by the 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 limit[2] of 0.01 mg/kg, i.e. the current MRL.

50. Recent monitoring of the wider UK food supply[3], has not identified γ-HCH in whole milk at a reporting limit of 0.0004 mg/kg, indicating that levels in infant formula produced from cows' milk produced in the UK are likely to be well below 0.01 mg/kg. Soya milk was last included in the UK pesticide monitoring programme in 2006. γ-HCH was not detected at a reporting limit of 0.002 mg/kg; nor was it detected at this reporting limit in soya pieces or tofu. This indicates that levels in soya-based infant formula are likely to be well below 0.01 mg/kg.

51. Much of the infant formula consumed in the UK is imported from Ireland (Public Health England, personal communication, 2014). A survey by the Food Safety Authority of Ireland in 2004 included six samples of infant formula (five of cows' and one of soya milk-based formula), and γ-HCH was not detected at a limit of quantification (LOQ) of 0.001 mg/kg of reconstituted formula. A total diet study carried out in Ireland during 2001-2005 included cows' milk and found no levels at or above the LOD of 0.0007 mg/kg (FSA Ireland, 2011). A monitoring programme conducted in Barcelona (Catalonia, Spain) during 2001-2006 that included infant formula, did not find γ-HCH in any of the 1,484 samples analysed (Fontcuberta et al., 2008), the LOQ being 0.01 mg.

52. A study in Huelva (Andalucia, Spain) measured levels of γ-HCH and total HCH (sum of α-, β- and γ-HCH) in milk formula reconstituted according to manufacturers' instructions. The mean levels were 0.021 and 0.022 mg/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.

53. A survey of the pesticide content of 25 infant formulae marketed in New Zealand was undertaken in 1996. It included a representative mixture of products, some imported and some manufactured in New Zealand. Approximately 140 pesticides including γ-HCH were assayed. γ-HCH was not detected with an LOD of 0.0002 mg/kg (Cressey and Vannoort, 2003).

Complementary food

54. Cereal-, fruit- and vegetable-based infant foods 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 limit of 0.01 mg/kg.

55. Recent monitoring by PRiF of the wider UK food supply has not identified residues of γ-HCH in any food commodities, which is consistent with its no longer being used as a pesticide and having a relatively short half-life of elimination in

[2] The reporting limit is defined as the lowest calibrated level employed during analysis for a pesticide residue. Quantification of lower levels would be unreliable.
[3] http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF

animals. For example, in the 2012 UK pesticide monitoring programme, γ-HCH was not identified in any of 3,657 samples of a wide range of agricultural commodities, processed foods and drinks (3,537 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; and were 0.01 mg/kg for most commodities, including fruits, vegetables, cereals, butter, cheese and olives. It therefore appears very unlikely that γ-HCH would be present in composite food products such as infant foods, at levels close to or above the MRL and reporting limit of 0.01 mg/kg.

56. There were no positive results for γ-HCH in 41 infant food samples tested in Ireland (Food Safety Authority of Ireland, 2004). The LOQ was 0.001 mg/kg. The samples included biscuits for infants, fruit-based infant food, vegetable/meat-based infant food, cereal-based infant food, and juices for infants and young children.

57. A survey of the pesticide content of 30 weaning foods available in New Zealand was undertaken in 1996. It included a representative mixture of products imported or manufactured in New Zealand. γ-HCH was not detected with an LOD of 0.0002 mg/kg (Cressey and Vannoort, 2003).

Exposure

58. Market basket studies performed between 1994 and 2003 in the Czech Republic, where HCHs were produced and used for a long time, indicated a decline in dietary exposure. The median daily γ-HCH exposure (population age group not specified) 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).

59. Biomonitoring data from Germany also indicate a decrease in exposure to γ-HCH. The third German Environmental Survey (GerES III) was conducted in 1998, using blood samples from 4,800 subjects aged 18-69 years, who were geographically representative of the German population (Becker et al., 2002). The arithmetic mean and the maximum values for all subjects were <0.1 and 4.7 µg/L respectively. Concentrations were above the LOQ (0.1 µg/L) in 5.2% of subjects. A further survey (GerES IV) carried out during 2003-2006, using blood samples from 1,063 children aged 7 to 14 from 150 randomly selected locations in Germany, found no subjects with levels above the LOQ of 0.076 µg/L (Schulz et al., 2009).

60. In calculating potential exposures from breast milk and infant formula, daily milk intakes of 800 mL (average consumer) and 1200 mL (high consumer) were assumed, as in other COT statements on the infant diet. Bodyweight data were obtained from the UK Dietary and Nutrition Survey of Infants and Young Children (DNSIYC) (DH, 2013), which indicated average values 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, a value of 5.9 kg for infants aged 0-3 months in an earlier survey (DH, 1994), was assumed for infants aged 0-4.0 months.

Breast milk

61. Estimates of exposures from breast milk were based on the geometric mean concentration (0.8 µg/kg fat, 0.028 µg/kg whole milk) of γ-HCH in breast milk from the most recent UK study presented in Table 2 (Kalantzi et al., 2004b) as an indicator of central tendency, and also on the maximum level (7.7 µg/kg fat, 0.27 µg/kg whole milk). It was assumed that the fat content of breast milk was 3.5 % (see Table 4). Given the trends observed since monitoring began in the 1970s, concentrations of γ-HCH in breast milk are expected to have decreased since 2001-2003 when the survey by Kalantzi et al. (2004b) was conducted. Thus, the values assumed are likely to overestimate current exposures.

Table 4. Exposure of infants to γ-HCH (µg/kg bw/day) from exclusive breastfeeding estimated for average and high consumption of milk.

Estimated γ-HCH concentration in whole breast milk (µg/kg)	Age in months (milk consumption per day)			
	0-4.0 (800 mL)	0-4.0 (1200 mL)	>4.0-6.0 (800 mL)	>4.0-6.0 (1200 mL)
Geometric mean - 0.028	0.0038	0.0057	0.0028	0.0043
Maximum - 0.27	0.037	0.055	0.028	0.042

Infant formula

62. In the absence of quantified measurements, potential exposures from infant formula were estimated using the LOQ for γ-HCH in reconstituted infant formula (0.001 mg/kg) in an Irish survey which did not detect the compound (FSA Ireland, 2011; see paragraph 51). From the summary reports provided by water companies in several regions of the UK, only four of 3,565 samples exceeded the typical LOD for γ-HCH (0.003 µg/L). If γ-HCH were present at 0.003 µg/L in water used to reconstitute infant formula, the exposure from the water would be up to approximately 0.0006 µg/kg bw day, which would have negligible impact on the total intake from reconstituted formula if the level in the formula were 0.001 mg/kg. Table 5 shows the exposures that might result from consumption of infant formula containing γ-HCH at 0.001 mg/kg.

Table 5. Theoretical maximum exposure of infants to γ-HCH (µg/kg bw/day) from exclusive feeding on cows' or soya milk-based infant formula.

γ-HCH concentration	Age in months (consumption volume per day)			
	0-4.0 (800 mL)	0-4.0 (1200 mL)	>4.0-6.0 (800 mL)	>4.0-6.0 (1200 mL)
< LOQ of 0.001 mg/kg	< 0.135	< 0.203	< 0.103	< 0.154

Given the levels reported in paragraph 45, the contribution from water used for reconstitution has not been added as it is likely to be extremely low.

Complementary foods

63. The average and 97.5th percentile of total solids consumed by 4-12 month old infants from the DNSIYC were estimated to be 39 and 78 g/kg bw/day respectively. No samples were found to contain γ-HCH at or above the MRL (0.01 mg/kg) in surveys carried out in the UK on cereal-, fruit- and vegetable-based foods, and other products containing egg, fish, meat or cheese to be consumed by infants. As recommended by US EPA (2000), in the absence of detected/quantified levels, a value of ½ MRL (0.005 mg/kg) was used to estimate potential exposures to γ-HCH. This gave values of 0.20 µg/kg bw/day for average consumers and 0.39 µg/kg bw/day for high level consumers.

Risk characterisation

64. Estimated exposures to γ-HCH from breast milk were below the TDI of 0.04 µg/kg bw, except that from high level consumption of breast milk containing γ-HCH at the maximum reported concentration in UK breast milk in 2001-3, which gave a minor exceedance. Given that levels in breast milk have been decreasing over time, these findings do not indicate a concern for the health of breastfed infants.

65. From the data that are available, it is not possible to exclude the possibility that the exposure of some infants to γ-HCH from infant formula could be five times the TDI of 0.04 µg/kg bw, and that from infant food some ten times the TDI. However, this is because of the limited sensitivity of the methods that have been used to look for γ-HCH in these foods, and it is likely that actual exposures are much lower. The available data indicate that exposure from water used to reconstitute infant formula is not a concern.

66. Estimated exposures from breast milk, infant formulae and infant foods are all substantially below the ARfD of 60 µg/kg bw/day established by JMPR.

α-HCH

Toxicokinetics

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

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

69. The metabolism of α-HCH involves dehydrogenation, dehydrochlorination and dechlorination, which may be followed by conjugation. Known metabolites of α-HCH include chlorophenols, chlorobenzenes, chlorocyclohexanes, dichlorophenylglutathione and dichlorophenylmercapturic acid (Kraus et al., 1975; Macholz et al., 1982a).

70. After intraperitoneal injection in rats, 40-80% of α-HCH was excreted in the urine and 5-20% in the faeces. No studies have been found that derived plasma elimination half-life values for α-HCH in humans or 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).

Toxicity of α-HCH

71. α-HCH is of low acute toxicity, with oral LD50 values in the region of 1,000 to 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).

72. Hepatotoxicity of α-HCH has been reported in many studies. For example, liver hypertrophy was reported at 10 ppm diet (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 ppm diet. The NOAEL was 2 ppm diet (equivalent to 0.1 mg/kg bw/day). Decreased body weight was reported at the highest dose (equivalent to 12.5 mg/kg bw/day) (Kuiper et al., 1985; cited in EFSA, 2005).

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

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

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

Genotoxicity

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

77. 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 pre-treatment with phenobarbitone. The

authors concluded that the findings suggested a non-genotoxic mechanism for the tumorigenic effect of α-HCH (see section on carcinogenicity below).

78. In a study by Sagelsdorff 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 were genotoxicity mediated by DNA-binding, as seen with carcinogens such as aflatoxin B1 and dimethylnitrosamine.

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

80. 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).

Carcinogenicity

81. α-HCH has been shown to cause liver tumours in multiple mouse strains, and also in rats, although rats were less sensitive than mice (IPCS, 1992; Integral Consulting, 2011a).

82. Initiation-promotion studies indicate that α-HCH is a tumour promoter. In a study by Masuda et al. (2001), rats were injected with 200 mg/kg bw diethylnitrosamine (DEN) followed by two weeks of basal diet, after which they were fed a 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. CYP3A2 protein expression was statistically significantly increased at dietary concentrations of 15 to 500 ppm. CYP2B1 protein expression was statistically significantly increased at 60 and 500 ppm. CYP1A1 was also investigated but did not produce a response at any of the doses investigated. At the highest dose level of 500 ppm, the CYP enzyme protein expression levels were 2B1 > 3A2 > 1A1. 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.

83. Similarly in a study by Puatanachokchai et al. (2006), rats were injected three times in one week with 100 mg/kw bw DEN, and were then given a diet containing 0.01 to 500 ppm α-HCH for 10 weeks. At 500 ppm α-HCH, CYP2C11/6, CYP2E1, CYP3A1/2 and NADPH-cytochrome P450 reductase protein expression was

increased by more >2-, 1.5-, 5- >2.5-fold, respectively. In contrast to the other CYP enzymes investigated, CYP2B1 was not detected in the control group, but was significantly increased by treatment with 50 and 500 ppm α-HCH. The number of GST-P-positive foci was statistically significantly increased at 50 and 500 ppm, and their 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 these studies at the feed concentration of 1 ppm was equivalent to 0.09 mg/kg bw/day, using the default conversion factor of 0.09 proposed by EFSA (2012) for subchronic rat studies.

84. Based on the available data for α-HCH, and by analogy to γ-HCH, the COT concluded that α-HCH is likely to be a non-genotoxic liver carcinogen in rodents, acting by CAR activation. This MOA for rodent liver tumour formation is considered not to be relevant to humans (Elcombe et al., 2014).

Observations in humans

85. A hospital-based case-control study in India found associations of breast cancer with 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 the breast cancer patients than in the controls. However, no adjustment was made for potentially important confounding factors such as breast feeding, number of children, occupation and body fat mass. Nor did the analysis take into account the levels of other organochlorines. Therefore it does not allow useful conclusions regarding associations with HCHs.

86. In a comparison of 18 boys undergoing surgery because of undescended testes (mean age 4.2 years) and 30 surgical controls (mean age 3.5 years), Hosie et al. (2000) found no associations between undescended testes and α-HCH levels in fat samples.

Allergy

87. No studies have been found relating α-HCH to the occurrence of allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

88. Table 6 summarises TDI and RfD values that have been proposed for α-HCH.

Table 6 Health-based guidance values proposed for α-HCH.

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

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

90. The RfD for α-HCH proposed by Integral Consulting (2011a) was based on the study by Masuda et al., (2001) on promotion of hepatocarcinogenicity in male rats (paragraph 82). The NOAEL was reported to be 0.1 mg/kg bw per day. An uncertainty factor of 300 (components of 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.

91. The COT concluded that it was not possible to endorse any of these values. The findings of Masuda et al. (2001) relating to tumour promotion were considered to be of uncertain human relevance, and there was insufficient information on the study used by RIVM (2001) to derive a TDI. The COT concluded that, because the toxicity of α-HCH has not been well characterised, 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 as a reference point (Kuiper et al., 1985; cited in EFSA, 2005), supported by the findings on tumour promotion in the studies by Masuda et al. (2001) and Puatanachoikchai et al. (2006).

Sources of α-HCH and occurrence levels

Drinking water

92. Reports from water companies across the UK summarise results from analyses of α-HCH. For example, in summary tables of the 2011 annual report of the Drinking Water Inspectorate (DWI, 2012) it was reported that the 99th percentile for

α-HCH was < 0.002 µg/L in 1172 samples taken from four areas: Wales, Severn Trent, Bristol and Wessex.

Breast milk

93. In a study that collected 92 samples of breast milk from 48 donors in the UK during 2001-2002, α-HCH was not found at a limit of detection of 0.01 mg/kg fat (Woolridge et al., 2004).

94. In the context of the 3rd WHO human milk field study (2000-2001), α-HCH was analysed in 16 samples of pooled human milk 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 0.002 to 0.006 mg/kg fat, the highest value coming from the Ukraine. These samples may have been affected by local sources of contamination, making them unrepresentative of the UK. α-HCH was not detected in other European samples at an LOD of 0.001 mg/kg fat.

Infant formula

95. Infant formula was last surveyed for pesticide residues in July-September 2009, when α-HCH was not detected at or above the reporting limit of 0.01 mg/kg (PRiF, 2010). Recent monitoring of the wider UK food supply has not identified residues of α-HCH in whole milk at a reporting limit of 0.002 mg/kg, which indicates that levels in cows' milk-based infant formulae are likely to be well below 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 limit of 0.002 mg/kg. Nor was it detected in soya pieces or tofu at this reporting limit, indicating that levels in soya-based infant formula are also likely to be well below 0.01 mg/kg.

96. As noted in paragraph 51, much of the infant formula consumed in the UK is imported from Ireland. α-HCH was not recorded in Irish surveys with LOQs of 0.001 mg/kg for reconstituted infant formula and 0.0007 mg/kg for cows' milk (FSA Ireland, 2011).

97. A study in Barcelona (Catalonia, Spain) during 2001-2006 on various food products including infant formula, did not find α-HCH in any of the 1,484 samples analysed with an LOQ of 0.01 mg/kg (Fontcuberta et al., 2008).

Complementary foods

98. Cereal-, fruit- and vegetable-based infant products, and other products to be consumed by infants, containing egg, fish, meat or cheese, 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 limit of 0.01 mg/kg.

99. Recent monitoring by PRiF of the wider UK food supply has not identified residues of α-HCH. This included 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, 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 for infants at levels close to or above the MRL and reporting limit of 0.01 mg/kg.

Exposure

100. In surveys in the Czech Republic (age group not specified), the median daily intake of α-HCH in 1994 was 4.3 ng/kg bw (Ruprich et al., 1995) and in 2002 it was 1.6 ng/kg bw (Ruprich et al., 2003).

101. Biomonitoring data in Germany also indicate a reduction in exposure to α-HCH. The third German Environmental Survey (GerES III) was conducted in 1998 using blood samples from 4,800 subjects aged 18-69 years geographically representative of the national population (Becker et al., 2002). The arithmetic mean and maximum values for all subjects were <0.1 and 0.4 µg/L respectively. Concentrations were above the LOQ (0.1 µg/L) in 1.7% of the subjects. A subsequent survey (GerES IV) carried out during 2003-2006 using blood samples from 1,063 children aged 7-14 years from 150 randomly selected locations in Germany, found no levels above the LOQ of 0.016 µg/L (Schulz et al., 2009).

102. Values for consumption and body weight used for the estimation of UK infant exposures to α-HCH are as described for γ-HCH (paragraph 60).

Breast milk

103. Since there are no quantified measurements of α-HCH relevant to breast milk in the UK, a worst case estimation was based on the LOD (1 µg/kg fat), which was not exceeded in those European countries contributing to the 3rd WHO human milk field study which were considered to be most relevant to the UK. These worst case exposures are presented in Table 7, and are calculated with the assumption that the fat content of breast milk is 3.5 %, and therefore the LOD of 1 µg/kg fat is equivalent to 0.035 µg/kg whole breast milk.

Table 7. Theoretical maximum exposure of infants to α-HCH (µg/kg bw/day) from exclusive breastfeeding estimated for average and high consumption of breast milk.

α-HCH concentration	Age in months (consumption volume per day)			
	0-4.0 (800 mL)	0-4.0 (1200 mL)	>4.0-6.0 (800 mL)	>4.0-6.0 (1200 mL)
< LOD of 0.035 µg/kg in whole breast milk	< 0.0047	< 0.0071	< 0.0036	< 0.0054

Infant formula

104. In the absence of quantified measurements, potential exposures from infant formula were estimated using the LOQ reported for reconstituted infant formula (0.001 mg/kg) in an Irish survey (FSA Ireland, 2011; see paragraph 55), which applied equally to α- and γ-HCH. Therefore the estimated exposure levels are the same as for γ-HCH (paragraph 62, Table 5).

Complementary foods

105. Exposures were estimated assuming concentrations at half the EU MRL (which is the same for all HCH isomers), in combination with average and 97.5th percentile values for total solids consumed by 4-12 month old infants from the DNSIYC survey (39 and 78 g/kg bw/day respectively). As for γ-HCH (paragraph 62), this gave estimated exposures of 0.20 and 0.39 µg/kg bw/day for average and high-level consumers respectively.

Risk characterisation

106. The MOEs calculated for potential exposures to α-HCH compared to the reference point for hepatotoxicity of 0.1 mg/kg bw/day are shown in Table 8.

107. Interpretation of these MOEs must take into account the uncertainties in both the toxicological reference point and the exposure estimates. An uncertainty factor of 100 is generally assumed appropriate to allow for inter- and intra-species differences in toxicokinetics and toxicodynamics, and for α-HCH there is additional uncertainty related to gaps in the toxicological database, such as an absence of published studies on reproductive toxicity. Against this, the derivation of exposure values from analyses of breast milk sampled about ten years ago, and from non-detectable levels in infant formulae and foods will have resulted in an overestimation of true exposures, and therefore will have tended to cause underestimation of the MOE.

108. The MOEs for maximal exposure from breast milk, of > 14,000, are large and not a concern.

109. The MOEs calculated for maximal predicted exposures from infant formula and infant food are smaller than for breast milk. However, when account is taken of

the uncertainties noted in paragraph 107, these do not indicate a concern for health. Furthermore there is evidence that exposures have been decreasing over time.

Table 8. MOEs (rounded) based on maximum potential exposures of infants to α-HCH compared to a reference of 0.1 mg/kg bw/day

Source	Consumption	Exposure (µg/kg bw/day)		
		0 - 4.0 months	>4.0 – 6.0 months	>6.0 –12.0 months
Breast milk	Average	> 21,300	> 27,800	N/A
	High level	> 14,100	> 18,500	
Infant formula	Average	>750	>950	N/A
	High level	>500	>650	
Infant food	Average	N/A	N/A	500
	High level	N/A	N/A	>250

N/A: not applicable

β-HCH

Toxicokinetics

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

111. 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 cross the blood-brain barrier less readily than other HCH isomers. Transplacental and lactational transfer has been reported (WHO-IPCS, 1992).

112. The metabolism of β-HCH involves dechlorination, dehydrogenation and dehydrochlorination (Macholz et al., 1982b). EFSA (2005) noted that in rodents β-HCH is metabolised at a slower rate than α- and γ-HCH, and that indirect evidence based on high residue levels indicates that this could also apply to other animal species and to humans. A number of studies in rodents cited in WHO-IPCS (1992) found that β-HCH was metabolised predominantly to 2,4,6-trichlorophenol, with fewer other chlorophenols reported. Metabolites are conjugated, mainly with glucuronic acid or sulphate (WHO-IPCS, 1992).

113. Rats fed β-HCH in the diet for 7 days excreted 70% of the dose within 28 days. One third of the excretion was as metabolites in the urine, suggesting that faecal excretion is more important for β-HCH than for α-HCH. A two-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 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. Half-lives of 22 days

for clearance from "internal organs" and 20 days from the brain have been reported in female rats. (WHO-IPCS, 1992).

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

115. β-HCH residues in human breast milk samples from 40 women who had lived a minimum of 5 years in Veracruz (Mexico), decreased during lactation from a mean of 95 (SD 60) µg/kg on the 4th day to 66 (SD 45) µg/kg on the 30th day (Waliszewski et al., 2009).

Toxicity of β-HCH

116. 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 acute toxicity were mainly related to stimulation of the nervous system (WHO-IPCS, 1992).

117. Hepatotoxicity has been reported for β-HCH in many studies. For example, toxicity was investigated in a 13-week study in rats given β-HCH at 0, 2, 10, 50, or 250 ppm feed (equivalent to 0.18, 0.9, 4.5 or 22.5 mg/kg bw/day according to EFSA (2012) default values for subchronic studies). There was a statistically significant increase in liver weight in both sexes with the 10 ppm dose. Hyalinization was observed in some males and females at doses of 2 ppm and higher, and hypertrophy of centrilobular cells at 250 ppm. CYP content was statistically significantly increased at 50 ppm and higher in males, and in all female dose groups, but because of technical limitations at the time when the study was conducted, the pattern of induction of CYP enzymes was not established. In the highest dose group, effects were observed on the thymus, testes and ovaries, with severe morbidity (Van Velsen et al., 1986a). The LOAEL from this study was at the lowest feed concentration of 2 ppm (0.18 mg/kg bw/day).

118. β-HCH has been reported to induce ataxia in acute and subchronic studies, with NOAELs of 5 mg/kg bw per day in rats and 19 mg/kg bw per day in mice. Other neurotoxic effects such as reduced tail nerve conduction velocity have been reported at higher doses (WHO-IPCS, 1992).

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

120. In a 2 generation-study by van Velsen (1986b, 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 ppm (equivalent to 0.18, 0.9 and 4.5 mg/kg bw/day) from weaning and

through to 12 weeks premating, and the F1 generation were similarly exposed through to weaning of the F_2 generation. Almost complete infertility was reported in the F1 generation at 50 ppm. At 10 ppm, precocious vaginal opening was reported in the second generation and complete infertility. No effects were reported in the group receiving 2 ppm. Another study by van Velsen et al. (1986a), based on a standard design for a 13 week regulatory study with 10 males and 10 females, indicated endocrine effects. In females, the absolute weight of the adrenal gland was statistically significantly increased at 2 and 10 ppm feed, and both absolute and relative weights at 250 ppm. An increase in absolute uterine weight was reported at 10 ppm feed. Other reported effects were atrophy of the testes, reduced size of seminiferous tubules and lower number of Leydig cells in males, these occurring with a NOAEL of 50 ppm feed, equivalent to 4.5 mg/kg bw per day.

Genotoxicity

121. β-HCH was not mutagenic in the spot test in *Bacillus subtilis* (Tanooka, 1977). A study investigating DNA binding in male mice found an extremely low level of binding in the liver 10 h after oral administration of β-[^{3}H]HCH i.p. (Sagelsdorff et al., 1983). Positive responses were reported in the micronucleus test in MCF-7 cells at 1 and 10 pM, but the lower concentration produced larger effects than the higher, which suggests that the positive result at this very low concentration was a chance finding. In the 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., 2004a). Overall, the COT concluded that the balance of evidence indicates that β-HCH is not mutagenic.

Carcinogenicity

122. Eight studies of carcinogenicity have been reported for β-HCH in rats and mice. While several of these studies had notable limitations, only one, by Thorpe and Walker (1973), suggested 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 according to the EFSA (2012) conversion factor of 0.15 for chronic mouse studies), resulting in liver tumours similar to those induced by γ-HCH.

123. Based on the available data on β-HCH, and by analogy to γ-HCH, the COT concluded that β-HCH is likely to be a non-genotoxic liver carcinogen in rodents, acting via CAR activation. This MOA for rodent liver tumour formation is considered not to be relevant to humans (Elcombe et al., 2014).

Observations in humans

124. In 2004, the 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".

125. Further studies have been published since 2004 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 women (n=23) and breast cancer patients (n=54) in Poland, reported higher levels of β-HCH in adipose tissue from the breast cancer cases. On the other hand, two studies carried out in Japan found no association between serum levels of β-HCH and breast cancer. During 2001-2005, Itoh et al. (2009) carried out a case-control study of breast cancer in Nagano Prefecture (Japan) based on 403 matched pairs. ORs (adjusted for various factors) and 95% CIs were estimated by conditional logistic regression. There was no increase in the risk of breast cancer among women with higher serum concentrations of β-HCH. A study by Iwasaki et al. (2008) followed up 22,466 women for 10.6 years, during which 114 cases of breast cancer were newly diagnosed. Two matched controls were selected for each case from within the cohort. Conditional logistic regression was used to estimate the OR for breast cancer according to cholesterol-adjusted organochlorine levels in plasma, but no association was found with β-HCH.

126. Most other studies investigating associations of β-HCH with risk of cancer have focused on non-Hodgkin lymphoma (NHL). Cantor et al. (2003) conducted a study of 74 cases and 174 controls nested in a community-based, US cohort. This had a strong design in that serum samples were obtained well before diagnosis (a median of 12 years). After adjustment for covariates including PCBs, the odds ratio in the highest quartile of concentrations relative to the lowest quartile was 1.5 (95% CI 0.5-4.3), but there was no consistent trend in risk with increasing concentration of β-HCH. A European case-control study including 174 cases and 203 controls (Cocco et al., 2008) and a US study of 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), which included 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, and the statistical significance was lost when adjustment was made for two other pesticides. A Canadian study by Spinelli et al. (2007) involving 422 cases and 460 controls found significant associations of β-HCH with NHL (OR for highest quartile of β-HCH 1.59, 95% CI 1.01-2.49), but it did not adjust for other pesticide residues or PCBs, with which β-HCH was moderately correlated.

127. Taken together, the inconsistent findings from epidemiological studies are not strongly suggestive of a hazard of NHL from β-HCH. However, inter-correlations of tissue concentrations of persistent organic pollutants make it difficult to demonstrate clear associations with any single compound. Also, small numbers of subjects have limited the statistical power of most studies to explore associations with subtypes of NHL.

128. Two US case-control studies have examined the relationship of β-HCH to cancers other than NHL or breast cancer, finding no association with either the incidence of endometrial cancer in a relatively small study of 90 cases and 90 controls (Sturgeon et al., 1998), or with risk of testicular germ cell cancer in a large study of 754 cases and 928 controls (McGlynn et al., 2008).

129. A number of studies have investigated potential associations between β-HCH and endometriosis. Most also investigated associations with γ-HCH and have been described earlier (paragraph 34). An investigation by Upson et al. (2013) in the USA found a statistically significantly higher risk of endometriosis with serum concentrations of β-HCH in the third as compared with the lowest quartile (OR 1.7; 95% CI: 1.0-2.8) but not for the highest vs. the lowest quartile (OR 1.3; 95% CI: 0.8-2.4). No significant dose-response relationship was seen. A study by Buck Louis et al. (2012) found that serum β-HCH was significantly associated with MRI-diagnosed endometriosis in a population sample of 127 women (adjusted OR per 1-SD increase in log-transformed β-HCH 1.72; 95% CI: 1.09-2.72). However, no association was observed between endometriosis and β-HCH in either serum or omental fat, in an operative group of 473 women undergoing laparoscopy or laparotomy. In a study by Lebel et al. (1998), 86 women with endometriosis and 70 controls, matched for the indication for laparoscopy, were recruited in the Quebec City area (Canada) during 1994. Plasma β-HCH concentrations did not differ significantly between cases and controls, and there was no significant linear trend in the adjusted odd ratios for endometriosis as β-HCH concentrations increased.

130. 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. This contrasts with two case-control studies in which measurements of β-HCH 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 subjects (49 of 50 cases, 41 of 43 controls) 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 1.00-1.07) 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, but no pesticides are well established causes of the disease.

131. 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 possible weight loss related to illness, which may have affected the studies in which samples were taken after diagnosis.

132. 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 boys undergoing surgery because of undescended testes and 30 surgical controls, and there were no statistically significant associations with β-HCH.

Allergy

133. A cross-sectional study by Miyake et al., (2011) investigated concentrations of β-HCH in breast milk and the occurrence of allergic disorders in a population sub-sample from a large cohort of pregnant Japanese women recruited through obstetric clinics (n = 124). The occurrence of wheeze and asthma was ascertained using questions from the European Community Respiratory Health Survey, while that of eczema and rhinoconjunctivitis was assessed using questions from the International Study of Asthma and Allergies in Childhood (ISAAC). Adjustments were made for age, smoking, family history of allergic disorders, and education. The prevalence of wheeze, asthma, eczema, and rhinoconjunctivitis was 9.7%, 4.8%, 13.7%, and 29.8%, respectively. The median concentration of β-HCH in breast milk was 28.3 ng/g lipid, (range, 4.5-253 ng/g lipid). The authors found no significant associations between concentrations of β-HCH and the prevalence of allergic disease.

134. No other studies were found relating β-HCH to allergy, atopic disease or hypersensitivity.

Health-based guidance values (HBGV)

135. Table 9 summarises TDIs and RfDs that have been proposed for β-HCH.

Table 9. Health based guidance values proposed for β-HCH.

Source of HBGV	**HBGV** µg/kg bw/day	**Critical effect and species NOAEL/LOAEL mg/kg bw/day**	Uncertainty factor	**Study selected to derive HBGV**
Health Canada (1992), cited in EFSA (2005)	Group TDI 0.3 for all HCH isomers	Unknown		
RIVM (2001)	TDI 0.02	Infertility in male rats NOAEL 0.02	1,000	Slooff and Mathijsen (1988)
Integral Consulting (2011b)	RfD 0.06	Hepatoxicity in rats LOAEL 0.18	3,000	Van Velsen et al., 1986a

136. The basis of the TDI set by Health Canada is not in the public domain. The RIVM TDI for β-HCH was based on an oral multi-generation study in rats reported by Slooff and Mathijsen (1988) with a NOAEL for infertility of 0.4 ppm in feed, equivalent to 0.02 mg/kg bw/day (no further details of this study are available). With an uncertainty factor of 1,000, the TDI was 0.02 µg/kg bw/day (the basis for the uncertainty factor was not stated). In 2001, RIVM (2001) re-evaluated the scientific evidence and confirmed its TDI.

137. 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 117). An uncertainty factor of 3,000 was applied to account for inter- and intra-species differences, use of a LOAEL rather than a NOAEL, use of a subchronic study, and database limitations, and this resulted in a proposed RfD of 0.06 µg/kg day.

138. The COT concluded that it was not possible to endorse any of these values, and that because the toxicity of β-HCH has not been well characterised, there was insufficient information to propose a TDI. Instead, it was more appropriate to apply a MOE approach using the LOAEL of 0.18 mg/kg bw/day based on centrilobular hypertrophy (paragraph 117) as a reference point. The study on reproductive toxicity by Van Velsen (1986b) was not reported in sufficient detail to be used.

Sources of β-HCH and occurrence levels

Drinking water

139. Reports from water companies across the UK include the results of analyses for β-HCH. For example, the 99th percentile for β-HCH was < 0.002 µg/L in 566 samples taken from two areas: Wales and Trent-Severn (DWI, 2012)

Breast milk

140. A temporal decline in the levels of β-HCH in breast milk is apparent from the scientific literature. Table 10 shows the concentrations of β-HCH in breast milk from studies in UK populations published since 1965.

Table 10. Reported concentrations of β-HCH in breast milk in the UK from studies 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.R.	N.D.	7	33	13 (A or G not specified)	100	1963-1964	Egan et al., 1965
102	220	N.R.	150	10	4400	7	80	1979-1980	Collins et al., 1982
-	-	-	-	-	-	5 (A or G not specified)	95	1984	MAFF, 1998
193	80	N.R.	60	<20	990	2	82	1989-1991	Dwarka et al., 1995
156	68[a]	N.R.	50	<8	750	1	36	1997-1998	Harris et al., 1999
92	<100	<100	<100	<100	<100	<3.5[b]	0	2001-2002	Woolridge et al., 2004
54	40	15	17	1.2	1500	1.4[b]	100[c]	2001-2003	Kalantzi et al., 2004b

A. mean. Arithmetic mean, G. mean. Geometric mean, Med. Median, Min. Minimum, Max. Maximum., N.R. Not reported.
[a] Confirmed as arithmetic mean by C Harris (personal communication)
[b] Estimated from arithmetic mean assuming 3.5% fat in whole milk
[c] Limits of detection appear to have been much lower in this study than in those conducted earlier (<0.001 mg/kg fat *vs.* 0.01 mg/kg fat)

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

142. Levels of β-HCH in breast milk in populations from other European countries and the United States that have been published since 1994 are shown in Table 11.

Table 11. Concentrations of β-HCH in breast milk samples from other European countries and the United States, published since 1994

Country (City/Region)	µg/kg milk fat	Years samples collected	Reference
Italy (average of Rome, Milan, Florence and Pavia)	Mean 130	1987	Larsen et al., 1994
Germany (North)	Median 200	1986-1997	Schade and Heinzow, 1998
	Median 50		
Spain	Mean or median? 240	1991	Hernandez et al. in Wong et al., 2002
Germany (Saxony)	Median 40; 95th centile, 7,970	1992-1993	Raum et al., 1998
Germany (Saxony)	Median 59	1992-1993	Schlaud et al., 1995
German (Saxony – Rural areas)	Median 45	1992-1993	Schlaud et al., 1995
Russia (Murmansk)	Mean 853	1993	Polder et al., 1998
Russia (Monchegorsk)	Mean 740	1993	Polder et al., 1998
Ukraine	Median 731; 90thcentile, 1,305	1993-1994	Gladen et al., 1999
Norway (Oslo)	Mean 14 mean	2000-2001	Polder et al., 2008
Norway (Tromsø)	Mean 10	2000-2001	Polder et al., 2008
Germany (North Rhine-Westphalia)	Mean 130	1984	P Fürst, personal communication to EFSA, 2005.
	Mean 20	2001	
North Germany	Median 11.6	2006	Zietz et al., 2008
USA (California)	Urban median 0.22; 75th centile, 0.24	2002-2007	Weldon et al., 2011
	Rural median 0.44; 75th centile, 0.52		

143. In a study of 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 duration of breast-feeding. Post-pregnancy body mass index was a significant positive predictor 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

144. Infant formula was last surveyed in the UK in July-September 2009 and β-HCH was not detected at or above the reporting limit of 0.01 mg/kg, which corresponded to the current MRL (PRiF, 2010). Recent monitoring of the wider UK food supply has not identified residues of β-HCH in whole milk at a reporting limit of 0.002 mg/kg, indicating that levels in cows' milk-based infant formulae 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 soya-based infant formula are likely to be well below 0.01 mg/kg.

145. As noted in paragraph 51 much of the infant formula consumed in the UK is imported from Ireland. β-HCH was not detected in the Irish surveys described in paragraph 51, with LOQs of 0.002 mg/kg for reconstituted infant formula and 0.0007 mg/kg for cows' milk (FSA Ireland, 2011).

146. A study in Barcelona (Catalonia, Spain) during 2001-2006 on marketed food products including infant formula did not find β-HCH in any of 1,484 samples with an LOQ of 0.01 mg/kg (Fontcuberta et al., 2008).

Complementary food

147. Cereal-, fruit- and vegetable-based products for infants 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 0.01 mg/kg.

148. Recent monitoring of the wider UK food supply has not identified residues of β-HCH. This included 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, 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 for infants, at levels close to or higher than the MRL and reporting limit of 0.01 mg/kg.

Exposure

149. In surveys in the Czech Republic (age group not specified) the median daily intake of β-HCH was 8.4 ng/kg bw in 1994 (Ruprich et al., 1995) and 2.1 ng/kg bw in 2002 (Ruprich et al., 2003).

150. Biomonitoring data in Germany also indicate a decline in exposures to β-HCH. The third German Environmental Survey conducted in 1998 (GerES III) analysed blood samples from 4,800 subjects aged 18-69 years, geographically representative of the national population (Becker et al., 2002). The arithmetic mean and maximum values for concentrations of β-HCH in all subjects were 0.16 and 7.8 µg/L respectively. Concentrations were above the LOQ (0.1 µg/L) in 34% of participants. A subsequent survey (GerES IV) carried out between 2003-2006, using blood samples from 1,063 children aged 7-14 years from 150 randomly selected locations in Germany, found levels above the LOQ of 0.04 µg/L in 76% of the subjects, with a median and 95th centile of 0.01 and 0.1 µg/L respectively (Schulz et al., 2009).

151. The values for consumption and body weight used for the estimation of exposures to β-HCH were as described for γ-HCH in paragraph 60.

Breast milk

152. Table 12 shows estimates of exposure to β-HCH based on the arithmetic mean value from the most recent UK study presented in Table 10 (i.e. 40 µg/kg milk fat, equivalent to 1.4 µg/kg whole milk assuming that the fat content of breast milk was 3.5 %), and also the maximum level (1500 µg/kg fat, equivalent to 52.5 µg/kg whole milk) of β-HCH in breast milk (Kalantzi et al., 2004b). The arithmetic mean was selected since it reflects the higher values reported by Kalantzi et al. (2004b), and is more conservative than the geometric mean. The arithmetic mean is considered to be a more plausible estimate of exposures than the maximum value since the distribution of the data and comparison with other studies indicated that the maximum value might not be reliable. The second highest reported level was 40 µg/kg milk fat, i.e. the same as the arithmetic mean. Furthermore, levels are expected to have decreased since 2001-2003, when samples were collected by Kalantzi et al. (2004b).

Table 12. Exposures of infants to β-HCH (µg/kg bw/day) from exclusive breastfeeding estimated for average and high consumption of milk**.**

β-HCH concentration estimated in whole breast milk (µg/kg)	Age in months (consumption volume per day)			
	0-4.0 (800 mL)	0-4.0 (1200 mL)	>4.0-6.0 (800 mL)	>4.0-6.0 (1200 mL)
Arithmetic mean - 1.4	0.19	0.29	0.14	0.22
Maximum - 52.5	7.12	10.68	5.39	8.08

Infant formula

153. In the absence of quantified measurements, potential exposures were estimated using the LOQ for β-HCH in reconstituted infant formula (0.002 mg/kg), in an Irish survey which did not detect the compound (FSA Ireland, 2011; see paragraph 51). From the summary reports provided by the water companies in several regions in the UK the 99th percentile for concentrations of β-HCH in drinking water was < 0.002 µg/L). If β-HCH were present at 0.002 µg/L in water used to reconstitute infant formula, the exposure from the water would be up to approximately 0.0004 µg/kg bw day, which would have a negligible impact on the total exposure from reconstituted formula if the level in the formula were 0.002 mg/kg. Table 13 shows the exposures that would result from consumption of infant formula containing β-HCH at the LOQ.

Table 13. Theoretical maximum exposures of infants to β-HCH (µg/kg bw/day) from exclusive feeding on cows' or soya milk-based infant formula .

β-HCH concentration	Age in months (consumption volume per day)			
	0-4.0 (800 mL)	0-4.0 (1200 mL)	>4.0-6.0 (800 mL)	>4.0-6.0 (1200 mL)
< LOQ of 0.002 mg/kg for reconstituted formula	< 0.27	< 0.41	< 0.21	< 0.31

The contribution from water used for reconstitution has not been added as it is likely to be extremely low (see paragraph 153).

Complementary foods

154. Potential exposures from complementary foods were estimated assuming concentrations at half of the EU MRL (which is the same for all HCH isomers), in combination with average and 97.5th percentile intakes of total solids consumed by infants aged 4-12 months in the DNSIYC survey (39 and 78 g/kg bw/day respectively). This gave estimated exposures to β-HCH of 0.20 and 0.39 µg/kg bw/day for average and high-level consumers, as for γ-HCH (paragraph 63).

Risk characterisation

155. The MOEs calculated for predicted exposures to β-HCH as compared with the reference LOAEL for hepatic centrilobular hypertrophy of 0.18 mg/kg bw/day are set out in Table 14.

156. Interpretation of these MOEs should take into account uncertainties both in the toxicological reference point and in the exposure estimates. A margin of 100 is generally assumed appropriate to allow for inter- and intra-species differences in toxicokinetics and toxicodynamics. For β-HCH, the reference point is a LOAEL rather than a NOAEL, and there are other gaps in the database, such as a lack of published studies on reproductive toxicity. On the other hand, there is uncertainty regarding the human relevance of the centrilobular hypertrophy that underpins the toxicological reference point, which means that the latter may be conservative. The assessment of exposures was based on breast milk sampled about ten years ago, and on non-detectable levels in infant formulae and foods, and this will have resulted in an overestimation of true exposures, and therefore will have tended to cause underestimation of the MOE.

157. The MOEs for breastfeeding infants were in the region of 600 - 1300 for occurrence levels at the arithmetic mean and do not indicate a concern for infant health. The MOEs at the maximum level in breast milk reported by Kalantzi et al. (2004b) might indicate a concern if this level occurred in breast milk currently. However, this maximum level was more than 35 times higher than the next measured level is therefore likely to be highly atypical of those that occur currently in the UK. In addition, it should be noted that the toxicological effects of β-HCH relate to long term exposures and lower MOEs during the limited period of breastfeeding may not be problematic.

158. The MOEs for exposure to β-HCH from infant formula and solid infant foods are > 450 and > 700 respectively. Taking into account the uncertainties noted in paragraph 156, these also do not indicate a concern for infant health. Furthermore there is evidence that exposures are decreasing over time.

Table 14. MOEs calculated from comparison of potential exposures of infants to β-HCH with the reference point of 0.18 mg/kg bw/day

Source	Consumption	0 – 4.0 months	>4.0 – 6.0 months	>6.0 – 12.0 months
Breast milk	Average (mean occurrence)	950	1300	N/A
	Average (maximum occurrence)	25	33	
	High level (mean occurrence)	600	800	
	High level (maximum occurrence)	16	22	
Infant formula	Average	>650	>850	N/A
	High level	>450	>600	
Infant food	Average	N/A	N/A	900
	High level	N/A	N/A	>450

N/A: not applicable

Overall conclusions

159. In the past, HCH products were used as pesticides. The pesticidal use of HCH products in which γ-HCH made up < 99% of all HCHs was banned in 1978. The use of lindane (> 99% γ-HCH) was banned in 2000. As γ-HCH remained in use until later than other HCH isomers, data on its toxicity and occurrence are more extensive than for the other isomers.

160. Levels of HCHs in food and published estimates of dietary exposures indicate a global reduction over time, consistent with the withdrawal of authorised uses.

161. Food items are periodically surveyed for the occurrence of pesticide residues, and measurements that exceed specified reporting limits are documented. In the most recent surveys in the UK, no measurements of HCH isomers above reporting levels have been found in infant formula or infant foods.

162. α-, β- and γ-HCH are rapidly and extensively absorbed after ingestion. The three isomers are distributed throughout the body and have been detected in liver, kidney, brain, muscle, ovaries and, more markedly, in adipose tissue. β-HCH does not readily cross the blood-brain barrier.

163. Metabolism of the HCHs involves dehydrogenation, dechlorination, hydroxylation and dehydrochlorination, which may be followed by conjugation with glucuronic acid, sulphate and glutathione. Biotransformation to trichorophenol followed by conjugation is a major pathway. Little information is available on the kinetics of elimination of any of the isomers in humans or animals..

164. HCHs induce CYP enzymes, particularly CYP2B.

165. HCHs have shown evidence of hepatocarcinogenicity in rodents. Based on observed induction of CYP2B, this is likely to be through a non-genotoxic mechanism involving activation of the constitutive androstane receptor, a mechanism which is considered not to be relevant to humans. There have been few studies concerning associations of HCHs with cancer in humans. The best studied cancer is Non-Hodgkin lymphoma (3 studies of γ-HCH, 5 studies of β-HCH), for which the evidence for an association with γ-HCH is limited and weak, and for β-HCH is conflicting.

166. Epidemiological studies of HCHs have indicated associations with increased risk of a number of neurological conditions and endocrine effects. However, limitations of these studies mean that it is not possible to draw conclusions on causality.

167. The toxic effects of γ-HCH include neurotoxicity and immunotoxicity, with evidence of immunotoxic effects seen at lower doses in animal studies. No epidemiological studies on immunotoxicity endpoints were identified. The COT concluded that a TDI of 0.04 µg/kg bw, established by the RIVM and based on immunotoxic effects, should be applied in considering infants' dietary exposures to γ-HCH.

168. Estimated exposures to γ-HCH from breast milk are expected to be below the TDI of 0.04 µg/kg bw, except if there were high consumption of breast milk containing the compound at the maximum reported concentration in UK breast milk in 2001-3, which would give a minor exceedance. Given that levels in breast milk have been decreasing over time, the COT does not consider that this represents a concern for the health of breastfed infants.

169. Estimated exposures to γ-HCH from infant formula and food are based on an absence of quantifiable measurements, and in theory, it is possible that the exposures of some infants from infant formula could be 5 times the TDI of 0.04 µg/kg bw, and those from infant foods approximately 10 times the TDI. However, in practice it is likely that exposures are substantially lower than these theoretical maxima. Available data indicate that exposure from water used to reconstitute infant formula is not a concern.

170. The toxicity of α- and β-HCH is less well characterised than that of γ-HCH. The COT concluded that the available information was insufficient to propose a TDI for either of these isomers, and that it was more appropriate to apply a margin of exposure (MOE) approach, taking into account uncertainties both in the toxicological database and in exposure estimates.

171. For α-HCH the MOEs compared to a reference point of 0.1 mg/kg bw/day based on hepatotoxicity do not raise concerns regarding the exposures of infants from breast milk, infant formula or infant foods.

172. For β-HCH, the MOEs compared to a reference point of 0.18 mg/kg bw/day based on centrilobular hypertrophy in rats do not indicate a health concern for infants

exposed from infant formula or infant foods. However whilst exposures at the average levels reported in breast milk do not present a concern, it is not possible confidently to rule out a risk if exposures were at the highest level of β-HCH reported in breast milk sampled in 2001-3.

173. Overall the COT concluded that this evaluation does not provide a basis for recommendations on the infant diet relating to HCHs, particularly as levels in food suggest a reduction in exposure over time. However continued monitoring of HCHs in breast milk, infant formula and food, with appropriately sensitive methods, would be useful to confirm that there are unlikely to be any risks.

COT Statement 2014/03
April 2014

ABBREVIATIONS

ADI	Acceptable Daily Intake
AOR	Adjusted Odds ratio
ARfD	Acute Reference Dose
ATSDR	Agency for Toxic Substances and Disease Registry
bw	Body weight
CAR	Constitutive androstane receptor
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
Defra	Department of Environment, Food and Rural Affairs
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 of the United Nations
GerES	German Environmental Survey
GST-P	Glutathione S-transferase placental enzyme
HBGV	Health based guidance value
HCH	Hexachlorocyclohexane
IPCS	International Program on Chemical Safety
ISAAC	International Study of Asthma and Allergies in Childhood
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD50	Lethal dose for 50% of the population
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantitation
MAFF	Ministry of Agriculture, Fisheries and Food
MCF-7	Human mammary carcinoma cells
MOA	Mode of action
MOE	Margin of exposure
MRI	Magnetic Resonance Imaging
MRL	Maximum Residue Level
NHL	Non-Hodgkins Lymphoma
NK	Natural killer cells
MOE	Margin of Exposure
NOAEL	No observed adverse effect level
OR	Odds 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
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
WHO	World Health Organization

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