

**OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN
ON A REQUEST FROM THE COMMISSION RELATED TO
DDT
AS AN UNDESIRABLE SUBSTANCE IN ANIMAL FEED**

Question N° EFSA-Q-2005-182

Adopted on 22 November 2006

SUMMARY

DDT was commercially introduced as an insecticide in the 1940s. Technical DDT contains 65 – 80 % *p,p'*-DDT. Other important constituents in the technical grade products are *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. The latter two compounds (along with their ortho, para analogues formed from *o,p'*-DDT) are also the major breakdown products in biological systems. Unless otherwise stated in this opinion, “DDT and related compounds” or sum of DDT refer to *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD. The main insecticidal activity can be attributed to *p,p'*-DDT. Moreover, DDT is used as an intermediate in the production of the pesticide dicofol and may occur as a major impurity in the final product. DDT was banned in many European countries for most uses in the early 1970s. The use of DDT as a pesticide has been very restrictive since 1981 and banned since 1986 in the EU. Although being banned in most countries worldwide, DDT is still used for vector control especially in areas with endemic malaria, and extended use was recently recommended by WHO for indoor residual spraying to control malaria.

Because of the lipophilic properties and persistence in the environment, DDT and related compounds are bioaccumulated and biomagnified along the food chain. DDT is included in the Stockholm convention on persistent organic pollutants (POPs)¹ and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP).

DDT is readily absorbed in humans and animals; the half-life for DDT varies from one month in rats to four years in humans. In animals and humans DDE is generally more persistent than DDT. DDT and related compounds are transferred to milk and egg and accumulate in domestic animals and fish. DDT has low acute toxicity to mammals and most bird species.

The main target organs are the nervous system and the liver. It also affects hormonal tissues, reproduction, fetal development and the immune system. DDT including *p,p'*-DDE and DDD cause tumours mainly in the liver of experimental animals and are mostly negative in

¹ http://www.pops.int/documents/convtext/convtext_en.pdf

genotoxicity studies. DDT is classified by IARC as possibly carcinogenic to humans (group 2B). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) derived a provisional tolerable daily intake (PTDI) for DDT of 0.01 mg/kg b.w.

In its evaluation of DDT, the CONTAM Panel examined occurrence data to assess the levels that are currently found in the environment and in food and feed. Feed materials of animal origin, especially fish derived products, are in general more contaminated than feed materials of plant origin. In feed samples of animal origin the metabolite *p,p'*-DDE normally represents more than 50 % of sum of DDT. A considerable lower contribution of *p,p'*-DDE to the sum of DDT may indicate recent use of DDT. Samples of plant origin are generally dominated by the parent compound *p,p'*-DDT. Feed commodities including fish derived products generally contain levels in the low µg/kg range and thus are far below those that have been found to cause adverse effects in fish and domestic animals. However, it can not be excluded that elevated levels may be found in feed commodities that originate from areas where DDT has recently been or still is used.

Despite its presence in the environment, many foodstuffs and animal feed, the data show a considerable decline of up to 90 % in human exposure to DDT and related compounds over the past three decades. Food of animal origin is the major source of human exposure and recent studies performed in some EU Member States indicate a mean dietary intake for adults and children of 5 - 30 ng/kg b.w. per day. This exposure level is more than two orders of magnitude below the PTDI of 0.01 mg/kg b.w.

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LIST OF ABBREVIATIONS AND ACRONYMS

ASE	Accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
B.w.	Body weight
CAS	Chemical Abstract Service
CEN	European Committee for Standardization
CLRTAP	Convention on long-range transboundary air pollution
DDA	2,2-bis(4-chlorophenyl) acetic acid
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDMU	1-chloro-2,2-bis(4-chlorophenyl) ethane
DDT	dichlorodiphenyltrichloroethane
ECD	Electron capture detection
EI	Electron impact
EMRL	Extraneous maximum residue limits
FAO	Food and Agriculture Organization
GPC	Gel permeation chromatography
HR	High resolution
IARC	International Agency on Research on Cancer
IPCS	International Programme on Chemical Safety
JMPR	Joint WHO/FAO meeting on pesticide residues
LD ₅₀	Dose that causes death among 50 % of treated animals
LOAEL	Lowest observed adverse effect level
MAE	Microwave assisted extraction
ML	Maximum level
MRL	Maximum residue level
MS	Mass spectrometry
NCI	Negative chemical ionization
NOAEL	No observed adverse effect level
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
PTDI	Provisional tolerable daily intake
SCAN	Scientific Committee on Animal Nutrition
SFE	Supercritical fluid extraction
SPE	Solid phase extraction
TDE	Synonym for DDD (dichlorodiphenyldichloroethane)
UNECE	United Nation Economic Commission for Europe
WHO	World Health Organization

BACKGROUND

1. General Background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed² replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition³.

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination (“early warning system”) and to take measures to reduce or eliminate the contamination (“pro-active approach”).

In particular the introduction of the principle of non-dilution is an important and far-reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful means to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)⁴.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003⁵ provides a comprehensive overview on the possible risks for

² OJ L140, 30.5.2002, p. 10

³ OJ L 115, 4.5.1999, p. 32

⁴ Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

⁵ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)

animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself and by the Standing Committee on the Food Chain and Animal Health that for several undesirable substances additional detailed risk assessments are necessary to enable a complete review of the provisions in Annex 1 of Directive 2002/32 EC.

2. Specific Background

DDT (dichlorodiphenyltrichloroethane) has been widely used as a pesticide to control insects in agriculture and insects that carry diseases such as malaria. DDE (dichlorodiphenyldichloroethylene) and DDD or TDE (dichlorodiphenyl-dichloroethane) are chemicals similar to DDT that contaminate commercial DDT preparations. DDE and DDD enter the environment as contaminant of the commercial DDT formulation or as breakdown product of DDT.

The use of DDT as a pesticide has been very restrictive since 1981 and banned since 1986 in the EU by Council Directive 79/117/EEC of 21 December 1978⁶ which prohibited the placing on the market and use of plant protection products containing certain substances.

EU legislation on maximum residue levels (MRLs) for pesticides is laid down in four Council Directives

- Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables⁷
- Council Directive 86/362/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on cereals⁸
- Council Directive 86/363/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on foodstuffs of animal origin⁹
- Council Directive 90/642/EEC of 27 November 1990 on the fixing of maximum residue levels for pesticide residues in and on certain products of plant origin, including fruits and vegetables¹⁰.

Until 1997, MRLs were fixed only for raw commodities. Council Directive 1997/41/EC of 25 June 1997¹¹ amending the above mentioned Directives, provided for a system applicable from 1 January 1999 to set MRLs in processed products and composite foodstuffs, based on the

⁶ OJ L 33, 8.2.1979, p. 36

⁷ OJ L 340, 9.12.1976, p.26

⁸ OJ L 221, 7.8.1986, p. 37

⁹ OJ L 221, 7.8.1986, p. 43

¹⁰ OJ L 350, 14.12.1990, p. 71

¹¹ OJ L 184, 12/07/1997, p. 33

MRLs fixed for the raw agricultural products. MRLs for processed products and composite foodstuffs are calculated on the basis of the MRL set for the agricultural commodity by application of an appropriate dilution or concentration factor and for composite foodstuffs MRLs are calculated taking into account the relative concentrations of the ingredients in the composite foodstuffs. As the consequence of the coming into force of Directive 1997/41/EC, the pesticide residue legislation applies also to animal feedingstuffs since 1 January 1999.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC¹² will replace the abovementioned Directives once it is applicable.

However some problems have currently been observed in implementing the pesticide residue legislation to animal feedingstuffs. The following problems have already been identified:

- compound feed is composed of a relatively high number of ingredients, of which several are processed products (by-products). It is not obvious to know what MRL is applicable to such compound feed as it involves many calculations and uncertainties and “unknowns” (processing factors),
- pesticide residue legislation does not yet cover products of marine origin which are regularly used in animal feed (no direct application),
- pesticide residue legislation does not yet cover products typically for animal feed (no food use) such as pastures, roughages, forages, fish oil and fish meal.

DDT (sum of DDT-, DDD- and DDE-isomers, expressed as DDT) is listed in the Annex to Directive 2002/32/EC.

¹² OJ L 70, 16.3.2005, p. 1

Table 1. The provisions on the maximum levels for DDT in the Annex to Directive 2002/32/EC compared with the provisions foreseen in the pesticide legislation (footnote 6 - 11).

Directive 2002/32/EC		EU-Pesticide residue legislation	
ML for DDT (sum of DDT-, TDE- and DDE-isomers, expressed as DDT), relative to a feedingstuff with a moisture content of 12 %.		MRL for DDT (sum of DDT-, TDE- and DDE-isomers, expressed as DDT) applicable to the product as marketed.	
Product	mg/kg	Product	mg/kg
Fats	0.5	Fruit and vegetables	0.05
Other feedingstuffs	0.05	Oilseeds	0.05
		cereals	0.05
		Meat (fat)	1.00
		Milk	0.04
		Eggs	0.05

TERMS OF REFERENCE

In accordance with Article 29 (1) a of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion on the presence of DDT in animal feed.

This scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of DDT for the different animal species of relevance (difference in sensitivity between animal species) above which
 - signs of toxicity can be observed (animal health / impact on animal health),
 - the level of transfer/carry over of DDT from the feed to the products of animal origin results in unacceptable levels of DDT or of its metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by DDT and the characterisation, insofar as possible, of the distribution of levels of contamination.
- assessment of the contribution of the different identified feed materials as sources of contamination by DDT

- to the overall exposure of the different relevant animal species to DDT,
 - to the impact on animal health,
 - to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates.
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ASSESSMENT

1. Introduction

4,4-Dichlorodiphenyltrichloroethane (4,4'-DDT, *p,p'*-DDT) was already synthesized in 1874 by Othmar Zeidler. In 1939 its excellent insecticidal properties were discovered by the Swiss chemist Paul Müller, who received the Nobel Prize in Physiology or Medicine in 1948 for this discovery. DDT was commercially introduced in 1942 by Geigy. In a first large scale operation in Europe an epidemic typhus could be confined in Naples in 1944. DDT was part of the field pack of the allied troops during World War II to combat i.a. malaria and head lice. Since the mid-1940s DDT was applied worldwide in large quantities due to its excellent properties to control insect pests on crop and forest lands, around homes and gardens, and for industrial and commercial purposes. DDT helped to almost completely eradicate malaria in Europe. The peak of DDT use was reached around 1960 where approximately 80,000 tons were sprayed annually. In 1962 Rachel Carson published her book "Silent Spring" in which she concluded that DDT and other pesticides had irrevocably harmed birds and animals and had contaminated the entire world food supply. Growing concerns about increased insect resistance, adverse environmental side-effects especially on wild birds, bioaccumulation and long-term toxicity in mammals led to severe restrictions in the late 1960s and finally resulted in a ban of DDT in a number of European countries in the early 1970s. Since 1986 it has been completely banned as a pesticide in the European Union but is still in use for vector control in those countries with endemic malaria. DDT is included in the Stockholm convention on persistent organic pollutants (POPs)¹³ and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP)¹⁴. Recently, WHO has announced¹⁵, nearly thirty years after its recommendation to phase out the widespread use of indoor spraying with DDT to control malaria, DDT will continue to play a major role in its efforts to fight the disease. WHO is now recommending the use of indoor surface spraying with DDT not only in epidemic areas but also in areas with constant and high malaria transmission, including throughout Africa.

DDT and especially its break down product DDE are ubiquitous in the environment.

¹³ http://www.pops.int/documents/convtext/convtext_en.pdf

¹⁴ <http://www.unece.org/env/lrtap/full%20text/1998.POPs.e.pdf>

¹⁵ <http://www.who.int/mediacentre/news/releases/2006/pr50/en/index.html>

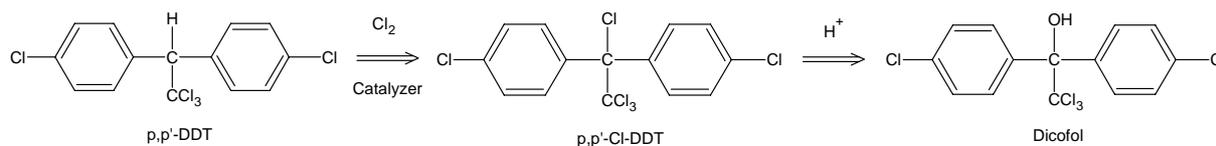
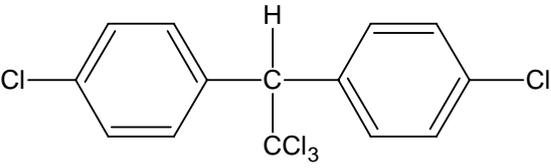
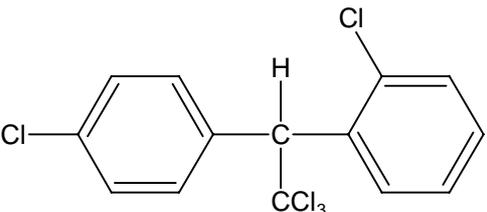
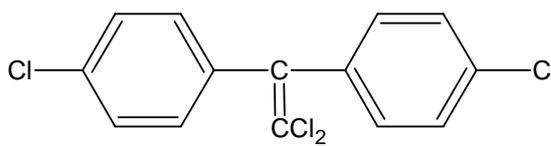
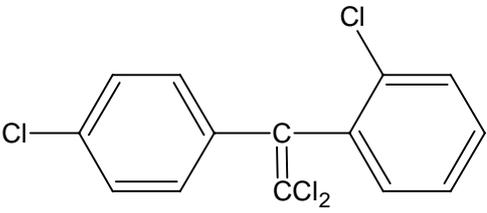
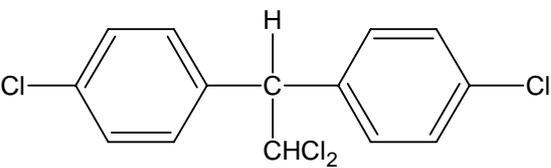
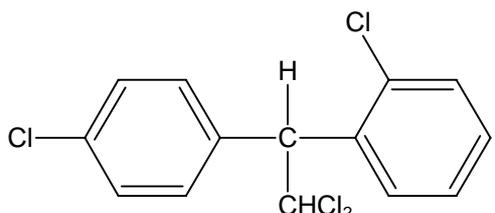


Figure 2. Synthesis of Dicofol from *p,p'*-DDT.

Analytical studies have shown that *p,p'*-DDT, *p,p'*-DDE as well as the intermediate 1,1,1,2-tetrachloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-Cl-DDT) may be present in technical grade dicofol (ATSDR, 2002). While in the EU, the amount of DDT and related compounds in dicofol is limited to 0.1 %, (Directive 79/117/EEC¹⁶), the analysis of 23 commercial dicofol formulation from 7 producers in China revealed average concentrations for *o,p'*-DDT, *p,p'*-Cl-DDT, *o,p'*-DDE, and *p,p'*-DDT of 11.4, 6.9, 4.4, and 1.7 %, respectively (Qiu *et al.*, 2005).

¹⁶ Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances. OJ L 033 , 08.02.1979, pp36–40.

Table 2. Chemical identity and properties of DDT and related compounds (adapted from ATSDR, 2002).

Structure	Name/Synonym	Properties
	1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane p,p'-DDT 4,4'-DDT	Melting point: 109°C Solubility (water): 0.025 mg/L (25°C) Log K _{ow} /Log K _{oc} : 6.91 / 5.18 Henry's Law const.: 8.4 x 10 ⁻¹ Pa m ³ /mol
	1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane o,p'-DDT 2,4'-DDT	Melting point: 74.2°C Solubility (water): 0.085 mg/L (25°C) Log K _{ow} /Log K _{oc} : 6.79 / 5.35 Henry's Law const.: 6.0 x 10 ⁻² Pa m ³ /mol
	1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene p,p'-DDE 4,4'-DDE	Melting point: 89°C Solubility (water): 0.12 mg/L (25°C) Log K _{ow} /Log K _{oc} : 6.51 / 4.70 Henry's Law const.: 2.12 Pa m ³ /mol
	1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethylene o,p'-DDE 2,4'-DDE	Melting point: no data Solubility (water): 0.14 mg/L (25°C) Log K _{ow} /Log K _{oc} : 6.00 / 5.19 Henry's Law const.: 1.82 Pa m ³ /mol
	1,1-dichloro-2,2-bis (p-chlorophenyl) ethane p,p'-DDD 4,4'-DDD TDE	Melting point: 109-110°C Solubility (water): 0.090 mg/L (25°C) Log K _{ow} /Log K _{oc} : 6.02 / 5.18 Henry's Law const.: 4.1 x 10 ⁻¹ Pa m ³ /mol
	1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane o,p'-DDD 2,4'-DDD	Melting point: 76-78°C Solubility (water): 0.10 mg/L (25°C) Log K _{ow} /Log K _{oc} : 5.87 / 5.19 Henry's Law const.: 8.3 x 10 ⁻¹ Pa m ³ /mol

1.2. Production, use and environmental fate

Production and use

DDT is a broad spectrum insecticide that was popular due to its effectiveness, long time action, relatively low acute mammalian toxicity, and low cost. DDT was first used as an insecticide starting in 1939 and widely used until about 1970 (Van Metre *et al.*, 1997). DDT was used during the Second World War to protect troops and civilians from the spread of malaria, typhus and other vector borne diseases. DDT has been broadly applied in agriculture to control insects on various kinds of crops and for the control of disease vectors. In 1972, 67 - 90 % of the total United States use of DDT was on cotton; the remainder was primarily used on peanuts and soybeans. DDT has been used extensively to eradicate forest pests, such as the gypsy moth and spruce budworm. It was used in the home as a mothproofing agent and to control lice. In some regions of the world where malaria poses a problem, DDT is sprayed onto the interior surfaces of homes to decrease the incidence and spread of the disease by controlling mosquitos (Attaran *et al.*, 2000; Roberts *et al.*, 2000). Not only is DDT a contact toxin for mosquitos, it is also a contact irritant and repellent. As such, DDT has been shown to be effective in controlling malaria by not only limiting the survival of the mosquito, but also decreasing the likelihood of an individual being bitten within the sprayed homes.

The total accumulative global usage of DDT has been estimated to be 2.6 million tonnes from 1950 to 1993 (Voldner and Li, 1995). In a review on DDT use in Europe from 1970 to 1995, Pacyna *et al.* (2003) estimated that the usage was largest in 1970 (27,900 tonnes) and decreased to 320 tonnes in 1993. Most of the usage in Europe took place in Eastern Europe as well as in Spain, Italy and France. Since 1986 DDT is completely banned as a pesticide in the European Union. Moreover, in 2003 DDT was banned in 60 non-EU countries. According to information provided to UNEP as part of the Stockholm Convention negotiations, DDT production continues in China and India for use in fighting malaria and other insect-borne diseases in over 25 countries (AMAP, 2004). However, this usage of DDT is declining in tropical countries, e.g. Mexico reduced its usage from 1800 tonnes in 1991 to 497 tonnes in 1997 and further reduction is planned (UNEP, 2002). The largest known current producer is India with approximately 7 tonnes per year.

The recent signals from WHO to promote indoor spraying with DDT as one main intervention to fight malaria could result in an increased production worldwide¹⁷.

p,p'-DDD was also used to a minor extent as an insecticide. *o,p'*-DDD (Mitotane) is used medically in the treatment of cancer of the adrenal gland (PDR, 1999). DDE has no commercial use.

Environmental fate

During the period when DDT was extensively used, a large source of DDT release to air occurred during agricultural or vector control applications. Emissions could also have resulted

¹⁷ <http://www.who.int/mediacentre/news/releases/2006/pr50/en/print.html>

during production, transport, and disposal. Because use of DDT was banned in many European countries for most uses in the early 1970s, primary release of DDT in recent years should be negligible in Europe.

In soil, most DDT is broken down slowly into DDE and DDD, generally by the action of microorganisms. The degradation pathways of DDT under aerobic and anaerobic conditions have been reviewed by Zook and Feng (1999) and Aislabie *et al.* (1997). Although technical grade DDT may also contain DDE and DDD as contaminants, practically all DDE and DDD found in the environment is a result of environmental breakdown of DDT.

In a study of the bioavailability of DDT, DDE, and DDD to earthworms (Morrison *et al.*, 1999) it was shown that the concentrations of DDT, DDE, DDD, and Σ DDT were consistently lower in earthworms exposed to these compounds that had persisted in soil for 49 years than in earthworms exposed to soil containing freshly added insecticides at the same concentration. The uptake percentages of DDT and its metabolites by earthworms were in the range of 1.30 – 1.75 % for the 49 year-aged soil, but were 4.00 – 15.2 % for the fresh soil (Morrison *et al.*, 1999).

There is abundant evidence that DDT gets into the atmosphere as a result of emissions or volatilization. The process of volatilization from soil and water may be repeated many times and, consequently, DDT may be transported over long distances in the atmosphere by what has been referred to as a ‘global distillation’ from warm source areas to cold polar regions (Bard, 1999; Bidleman *et al.*, 1992; Goldberg, 1975; Ottar, 1981; Wania and MacKay, 1993). As a result, DDT and its metabolites are also found in arctic air, sediment, and snow with substantial accumulations in animals, marine mammals, and humans residing in these regions (Anthony *et al.*, 1999; Harner, 1997).

When in the atmosphere, about 50 % of DDT will be found adsorbed to particulate matter and 50 % will exist in the vapour phase (Bidleman, 1988). A smaller proportion of DDE and DDD is adsorbed to particulate matter. Vapour-phase DDT, DDE and DDD react with photochemically-produced hydroxyl radicals in the atmosphere; their estimated half-lives are 37, 17, and 30 hours, respectively.

DDT is removed from the atmosphere by wet and dry deposition and diffusion into bodies of water. The largest amount of DDT is believed to be removed from the atmosphere in precipitation (Woodwell *et al.*, 1971). DDT, DDE, and DDD are highly lipid soluble, as reflected by their log octanol-water partition coefficients (log K_{ow}) (see also Table 2). This lipophilic property, combined with an extremely long biological half-life is responsible for its high bioconcentration in aquatic organisms (i.e., levels in organisms exceed those levels occurring in the surrounding water).

Sediment is the sink for DDT released into water. There it is available for ingestion by organisms, such as bottom feeders. Reich *et al.* (1986) reported that DDT, DDE, and DDD were still bioavailable to aquatic biota in a northern Alabama River 14 years after 432 – 8000 tonnes of DDT were discharged into the river.

Generally the northern hemisphere is more contaminated than the southern hemisphere as revealed by levels in globally distributed species like marine mammals where samples from South Africa, Australia and western South America show invariably low concentrations of DDT and related compounds. On the other hand, eastern and western temperate North Atlantic, the Caribbean Sea, the waters around Japan, the tropical and temperate belts of the Indian and Pacific Oceans show relatively high levels of the same compounds (O'Shea and Brownell, 1994; Aguilar *et al.*, 2002). Notably higher concentrations of DDT and related compounds were observed in populations of marine mammals in the Mediterranean Sea and off California (harbour porpoises and bottlenose dolphins). Additionally, studies on the deep-sea living black scabbardfish (*Aphanopus carbo*) in the Atlantic show a continuous (tenfold) increase in concentrations of DDT and related compounds along a transect from Rockal, west of Ireland, down to Madeira (Mormede and Davies, 2003).

This finding is in line with results from global monitoring of POPs in air which shows that elevated concentrations of DDT and related compounds are most frequently found in Europe. Globally the highest recently reported concentrations of *p,p'*-DDE in the air are from Las Palmas (Canary Islands) and California. *p,p'*-DDT, on the other hand, was only reported in samples from Manila, Philippines, Kuwait City, and surprisingly from Malin Head, Ireland (Pozo *et al.*, 2006).

1.3. Toxicology in laboratory animals and hazard assessment for humans

DDT has been evaluated several times by various international bodies within WHO (WHO-IPCS, 1979; 1989; FAO/WHO 1985; IARC, 1991; FAO/WHO, 2001) and by the Agency for Toxic Substances and Disease Registry (ATSDR, 2002). In many studies, particular the old ones, it was not specified whether technical grade or pure *p,p'*-DDT was used. In the following the chemical tested is specified. If not specified in the reports or when summary statements are cited, only the term DDT is used.

The main acute effect of DDT is perturbations of ion transport in neuronal membranes leading to potentiation of transmitter release and central nervous system excitation. DDT has relatively low acute toxicity in humans with non-fatal doses up to 285 mg/kg. The oral acute toxicity is comparable in experimental animals, in rats (LD₅₀ 113 - 800 mg/kg per day), in mouse (LD₅₀ 237 - 300 mg/kg per day), guinea pig (LD₅₀ 400 mg/kg per day) and rabbit (LD₅₀ 300 mg/kg per day) (ATSDR, 2002).

Signs of acute toxicity are from the nervous system. Both central and peripheral, are affected to some degree. In animals, DDT can produce hyperexcitability, tremor, ataxia, and finally epileptiform convulsions. Humans have experienced prickling in the tongue and periorally, paraesthesia, nausea, dizziness, confusion, headache, malaise, and restlessness as well as rashes (FAO/WHO, 2001; ATSDR, 2002; Beard, 2006).

At lower doses the liver is the major target organ. DDT induces microsomal enzymes in all species tested. Only in some rodents (for prolonged periods at doses above 2 and 5 mg/kg

b.w. in mice and rats, respectively) does the endoplasmic reticulum increase so much that the entire liver cell enlarges and granules that are normally scattered throughout the cytoplasm are displaced to the margin of the cell, which is accompanied by a moderate increase in fat droplets. In male Fischer 344/NCr rat liver the potency for CYP2B induction appeared to be similar on the basis of the molar serum concentration for *p,p'*-DDT (98 % purity), *p,p'*-DDE (99 % purity), and *p,p'*-DDD (99 % purity) (Nims *et al.*, 1998). In female Wistar rat liver, which normally does not express CYP3A, technical-grade DDT (80 % *p,p'*-DDT; 20 % *o,p'*-DDT) strongly induced this enzyme, whereas for males no significant induction was seen. The effects of technical grade DDT on CYP2Bs and associated enzymes in rats indicate that males have a lower threshold of induction than females. Whether a similar sex difference operates in humans is not known (Sierra-Santoyo *et al.*, 2000). No changes in liver function were observed in workers exposed to 0.05 - 0.25 mg/kg b.w. per day (FAO/WHO, 2001).

A variety of effects on humoral- and cell mediated immune responses in rabbits and rodents caused by DDT have been reported (FAO/WHO 2001).

Effects in humans were reported reported in a cross sectional study (Karmaus *et al.*, 2005).

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1.3.1. Long-term studies of toxicity and carcinogenicity and observations in humans

Animals

Chronic exposure to DDT in mice, rats and monkeys causes liver toxicity including liver tumours. In cynomolgus and rhesus monkeys exposed to 20 mg *p,p'*-DDT/kg b.w. per day for 130 months (Takayama *et al.*, 1999) fatty changes in the liver were observed in 13 of 24 and 5 of 17 individuals in the treated and the control groups, respectively.

In a number of long-term studies in mice oral doses of DDT (doses usually above 7.5 mg/kg b.w.) caused liver-cell tumours, including carcinomas, in animals of each sex and hepatoblastomas in males. In other studies increased incidences of lung carcinomas and malignant lymphomas were observed. Oral administration of DDT (25 - 40 mg/kg b.w.) to rats increased the incidence of liver tumours in both sexes. In hamsters, DDT was administered orally at doses similar to or higher than those found to cause liver tumours in mice and rats, some increase in the incidence of adrenocortical adenomas was observed. Hence, DDT is considered adequately tested for carcinogenicity in mice, rats and hamsters. *p,p'*-DDE, has been tested for carcinogenicity by oral administration in mice and hamsters and produced liver tumours in all both species and sexes. DDD increased the incidence of liver tumours in male mice and of lung tumours of both sexes. Also thyroid tumours were observed in male rats (FAO/WHO, 2001).

p,p'-DDT was administered orally to 13 cynomolgus and 11 rhesus monkeys at a dose of 20 mg/kg b.w. per day for 130 months (Takayama *et al.*, 1999; Tomatis and Huff, 2000) (see above). A control group of 17 monkeys received corn oil. The two cases of malignant tumour detected in the treated group were a metastasizing hepatocellular carcinoma in a 233 month-old male and a well-differentiated adenocarcinoma of the prostate in a 212 month-old male. Benign tumours detected in the treated group included three cases of leiomyoma. No tumours were found in the control group of 17 monkeys. In addition two cases of intraductal hyperplasia of the breast in the *p,p'*-DDT-treated females, but not in the control group were observed. Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (FAO/WHO, 2001) could not reach a conclusion about the evidence for carcinogenicity of DDT in monkeys on the basis of this 130-month study with one dose.

Humans

Many epidemiological studies have been conducted on the relationship between environmental and occupational DDT exposure and human cancer. Variable results have been obtained with regard to lung cancer among *p,p'*-DDT production workers (IARC, 1991). In other studies increased risk of lymphatic and haematopoietic, pancreas (particularly with heavy occupational exposure) and liver cancer have been reported, but inconsistencies among studies, confounding by exposure to other pesticides and limitations in study size, exposure assessment and also study design preclude definitive conclusions (IARC, 1991; ATSDR, 2002; Cocco *et al.*, 2005). In a recent cancer mortality study 4552 male workers exposed to DDT during antimalarial operations in 1946 to 1950 were followed from 1955 to 1999 (Cocco *et al.*, 2005). A major strength in this study was the available external exposure information based on the occupation at the time of the antimalarial operations. The authors found little evidence for a link between occupational exposure to *p,p'*-DDT and mortality from any of the cancers previously suggested to be associated with DDT exposure. The numbers for some cancers, i.a. pancreatic cancer, were, however, relatively small, which limited the ability to identify smaller risks.

Several investigators have compared serum or tissue levels of DDT and/or DDE among individuals with and without cancer, with inconsistent results (IARC, 1991; ATSDR, 2002). Particularly studies on levels of *p,p'*-DDE and other DDT metabolites in serum or tissues and breast cancer have shown conflicting results. López-Cervantes *et al.* (2004) recently performed a meta-analysis of 22 studies and found strong evidence against the alleged relationship between *p,p'*-DDE and breast cancer risk.

1.3.2. Genotoxicity

Although there are conflicting data with regard to some genotoxicity end-points (i.a. induction of aneuploidy, dominant lethality in insects and chromosomal aberration in mammalian cells), in most studies, DDT (isomer not specified by IARC, 1991) did not induce genotoxic effects in rodent or human cell systems nor was it mutagenic to fungi or bacteria. *p,p'*-DDE weakly induced chromosomal aberrations in cultured rodent cells and mutation in mammalian cells and insects, but not in bacteria. In most studies, neither *p,p'*-DDD nor *o,p'*-DDD induced

genetic effects in short-term tests *in vitro* (IARC, 1991). *p,p'*-DDT (5.5 mg/kg b.w. intraperitoneally) induced structural chromosomal aberrations in spleen cells of mice (Amer *et al.*, 1996).

Increased levels of chromosomal aberrations and sister chromatid exchange have been observed in peripheral lymphocytes of small groups of workers exposed to DDT in addition to several other pesticides (Rupa *et al.*, 1988; 1989; 1991). No data were available on the genetic and related effects of metabolites of DDT in humans (IARC, 1991; FAO/WHO, 2001; ATSDR, 2002).

The Panel noted that DDT including *p,p'*-DDE and DDD are mostly negative in genotoxicity studies.

1.3.3. Intercellular communication and other biochemical effects

DDT inhibited gap-junctional intercellular communication in rodent and human cell systems and reduced gap-junctional areas in rat liver cells *in vivo*. In cultured rodent cells *p,p'*-DDE and *p,p'*-DDD inhibited gap-junctional intercellular communication (FAO/WHO, 2001; IARC, 1991).

Recently, evidence has been provided that DDT and its metabolites can also stimulate activator protein-1 (AP-1)-mediated gene expression through activation of the p38 mitogen activated protein kinase (MAPK) signaling cascade (Frigo *et al.*, 2004). DDT induces both the expression of the death ligand TNF- α and apoptosis through a p38 MAPK-dependent mechanism (Frigo *et al.*, 2005).

1.3.4. Reproductive/developmental toxicity

Neurodevelopmental studies were conducted in the 1-year-old infants from the population of a rural village of 5000 inhabitants in the vicinity (1 km) of an organochlorine compound factory (Flix, Catalonia, Spain) (Ribas-Fitó *et al.*, 2003). DDT was manufactured during some periods until 1971. In adults high concentrations of hexachlorbenzene were encountered whereas DDE levels in serum (4.6 ng/ml) were not higher than expected (Sala *et al.*, 1999). Cord serum levels of DDE did not differ from nearby villages (Sala *et al.*, 2001). Prenatal exposure to *p,p'*-DDE was associated with a delay in mental and psychomotor development at 13 months of age, whereas prenatal exposure to HCB had no effect on child neurodevelopment (Ribas-Fitó *et al.*, 2003). Recently this cohort together with a cohort from Menorca, where DDT exposure was higher, was followed up to the age of 4 years. Children, whose DDT concentrations in cord serum were > 0.20 ng/ml, had decreases in the verbal scale and in the memory scale when compared, with children whose concentrations were < 0.05 ng/ml. Stronger associations were seen among girls. No significant associations were seen for DDE (Ribas-Fito *et al.*, 2006a).

In a prospective cohort of 1712 children born between 1959 and 1996 decreased height in children up to seven years of age was associated to prenatal exposure to *p,p'*-DDE, among those showing the highest prenatal concentrations ($\geq 60 \mu\text{g/L}$) (Ribas-Fitó *et al.*, 2006b).

Humans

Few data are available on reproductive effects in humans, and these show no correlation between exposure to DDT and stillbirth, miscarriage, or premature rupture of foetal membranes and neurodevelopmental effects (FAO/WHO, 2001). In a recent study from Mexico 116 men aged 27 living in a malaria endemic area where DDT was sprayed until year 2000 in a cross-sectional study showed effects both on sperm motility parameters and sperm morphology which were positively correlated to plasma levels of *p,p'*-DDE (De Jager *et al.*, 2006).

In a prospective cohort from the USA of 1712 children born between 1959 and 1996, decreased height in children up to seven years of age was associated with prenatal exposure to *p,p'*-DDE, among those showing the highest prenatal concentrations ($\geq 60 \mu\text{g/L}$) (Ribas-Fitó *et al.*, 2006b)

1.3.5. Hormonal effects

The results of competitive binding assays showed that *o,p'*-DDT, *o,p'*-DDD, *o,p'*-DDE, and *p,p'*-DDT all bind to the human estrogen receptor and *o,p'*-DDT with the strongest affinity. Binding affinities of these compounds were approximately 1000-fold weaker than that of estradiol. *p,p'*-DDT and *o,p'*-DDE showed estrogenic activity both *in vitro* and *in vivo*, and they also bind to the androgen receptor. The *p,p'*-DDE, hydroxy-DDE and partly *o,p'*-DDT act as an antiandrogen and inhibit 5-dihydrotestosterone-induced transcriptional activation (FAO/WHO, 2001; Hartig *et al.*, 2002; Schrader and Cook, 2000).

3-Methylsulfonyl-DDE, which is a persistent but minor DDT metabolite in rats, mice and humans, is a potent adrenal toxicant in mice. This compound can be transported to the fetus through the placenta and to the offspring via mothers milk. Treatment of mice with a single dose of 3 mg/kg of 3-methylsulfonyl-DDE resulted in covalent binding of the compound to proteins followed by mitochondrial destruction in the adrenal zona fasciculata (Lund *et al.*, 1988). The binding and damage probably results from CYP11B activation in adrenal mitochondria (Jonsson *et al.*, 1991; 1995).

1.3.6. Evaluation and classification

IARC (1991) classified DDT, based on inadequate evidence for carcinogenicity in humans and sufficient evidence in experimental animals as possibly carcinogenic to humans (Group 2). JMPR (FAO/WHO, 2001) derived a provisional tolerable daily intake (PTDI) of 0.01 mg/kg b.w. on the basis of the lowest NOAEL of 1 mg/kg b.w. per day for numerous developmental effects in rats as summarised by ATSDR (ATSDR, 1994).

Current human exposure in the EU, where the use of DDT is banned, is mainly due to residues in food and in the form of DDE. The evaluations made by IARC and JMPR are based mainly on experiments with DDT, pure or technical form (for chemistry see 1.1), and a few studies on related compounds. DDT is metabolised to related compounds and it is not possible to determine which are responsible for the effect. Although the insecticidal activity and also hormonal effects varies between *p,p'*-DDT and the other compounds, for several toxic end points, e.g. in the liver, effects similar to those of DDT are also seen following exposure to DDE and DDD. Hence, it is reasonable to take the observed PTDI for DDT as sum of DDT (which includes *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD).

2. Methods of analysis

During the synthesis of the active insecticide *p,p'*-DDT considerable concentrations of *o,p'*-DDT may be formed. In addition, *p,p'*-DDD, *o,p'*-DDE and *p,p'*-DDE are all impurities in technical grade *p,p'*-DDT as well as metabolites. Consequently, besides *p,p'*-DDT the analysis of feed and food samples should also cover these compounds. In contrast, there seems no need to include methylsulfonyl-DDE into the analysis of feed, because this metabolite is a minor one except for certain marine mammals, as seals and polar bears (Norström, 2006) which are not important constituents of feed.

According to Article 11 of Regulation No 882/2004¹⁸ analysis methods used in the context of official controls shall comply with relevant Community rules or, (a) if no such rules exist, with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation; or, (b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

Contrary to a number of other undesirable substances, no fixed analytical methods are prescribed by the European Commission for the determination of DDT and related compounds in animal feed. Multi-residue procedures for PCBs and pesticides including DDT, DDE and DDD isomers using HRGC/ECD and HRGC/MS in animal feeding stuffs are currently elaborated by the Technical Committee CEN/TC 327 “Animal feeding stuffs – methods of sampling and analysis” of the European Committee for Standardization (CEN, 2005). The limits of quantification for the DDT, DDE and DDD isomers by applying HRGC-ECD and HRGC-MS are each given as 2.0 and 0.5 ng/g, respectively.

A number of other well-proven, validated multi-residue methods are available for the quantitative determination of DDT and related compounds in various environmental matrices, including food, feed and other biological specimens. Depending on type of feed material, whether being of plant or animal origin, extraction as well as the extent of necessary

¹⁸ Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. OJ L 165, 30/04/2004 P. 0001 – 0141.

subsequent clean up steps may differ considerably. While after grinding solid materials are commonly extracted with boiling organic solvents using conventional Twisselmann, Soxhlet, accelerated solvent extraction (ASE) or microwave assisted extraction (MAE) procedures or by supercritical fluid extraction (SFE), liquid samples are mostly extracted by liquid/liquid partitioning. Co-extracted fat and other compounds which potentially may disturb the determination of DDT and related compounds can be removed by gel permeation chromatography (GPC) or by adsorption chromatography on various solid phase extraction (SPE), such as Florisil or alumina.

Clean up procedures that involve concentrated sulphuric acid and/or alkaline saponification must be avoided when analysing for DDT and metabolites. Alkaline saponification of extracts leads to partial conversion of DDT to DDE. The latter compound may also be formed during the treatment of extracts with sulphuric acid if dicofol, an authorized organochlorine acaricide that is structurally similar to DDT, is present in the sample.

Due to the high electro negativity caused by the four or five chlorine atoms of DDT and its related compounds, high-resolution gas chromatography with electron capture detection (HRGC/ECD) is the analytical method widely used not only to differentiate between the various analogues and metabolites of DDT but also to separate them from possible interfering co-extractants.

An efficient separation of DDT and its related compounds from other interfering substances, such as other organochlorine pesticides and polychlorinated biphenyls (PCBs) is especially important when using HRGC/ECD. The gas chromatographic separation on two capillary columns of different polarity in routine monitoring programmes is therefore mandatory. Potential co-elution problems can also be overcome by applying combined high resolution gas chromatography/mass spectrometry (HRGC/MS) either in the electron impact (EI) or negative chemical ionization (NCI) mode. The latter ionization technique is exceptionally sensitive and allows the determination of DDT down to the femtogram (10^{-15}) range. Besides increased selectivity, mass spectrometric methods in general offer the possibility of performing the analyses by isotope dilution using ^{13}C -labeled internal standards. Because these compounds can be added to the samples at the very beginning of the analytical determination and behave as the native analytes, they allow a valuable overview on the losses during the analytical procedure and thus significantly increase the accuracy of the results.

Successful participation in intercomparison exercises is a prerequisite for a laboratory to be able to provide results of necessary quality. Interlaboratory studies have been conducted for organochlorine pesticides since 1969 without noticeable improvement in coefficient of variation between laboratories (de Boer and Law, 2003). There are several reasons for this lack of improvement during the last 30 years. One of the main reasons is probably a decrease in concentrations in the tested materials. Recent reports on results obtained in intercomparisons among marine laboratories show that analysis of DDT and related compounds is still difficult for many laboratories (Carvalho *et al.*, 1999; Villeneuve *et al.*, 2004; de Boer and Law, 2003), especially for *p,p'*-DDT where only about one fourth of the laboratories can provide satisfactory data for biota at a level of 10 $\mu\text{g}/\text{kg}$ (Wells and de Boer,

2006). Results for *p,p'*-DDE in intercomparisons exercises on marine biota are however of much better quality than for the other DDTs, probably due to higher levels of DDE in the samples. Additionally, the currently available methods do not produce very accurate data when the analytes are below 1 µg/kg (de Boer and Wells, 1997; de Boer and Law, 2003) but the use of targeted analysis for only DDT and related compounds, preferably by the use of GC-MS, could undoubtedly improve the overall performance in intercomparisons (de Boer and Law, 2003). Therefore, increased efforts for the improvement in analytical performance is highly recommended.

3. Statutory limits

DDT was banned in many European countries for most uses in the early 1970s. The use of DDT as a pesticide has been very restrictive since 1981 and banned since 1986 in the EU by Directive 79/117/EEC¹⁹ which prohibited the placing on the market and use of plant protection products containing certain substances.

DDT is listed in the Annex to Directive 2002/32/EC on undesirable substances in animal feed²⁰ which on 1 August 2003 replaced Directive 1999/29/EC on the undesirable substances and products in animal nutrition²¹. The maximum levels which apply to the sum of DDT-, DDD (TDE see Table 2) - and DDE-isomers, expressed as DDT each pertain to a feedingstuff with a moisture content of 12 %. See also specific background.

The Codex Alimentarius Commission adopted “extraneous maximum residue limits” (EMRL) for DDT (defined as sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-TDE (DDD)) for several commodities of plant and animal origin²². An EMRL refers to a pesticide residue or a contaminant arising from environmental sources (including former agricultural uses) other than the use of a pesticide or contaminant substance directly or indirectly on the commodity. It is the maximum concentration of a pesticide residue or contaminant that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food, agricultural commodity, or animal feed.

4. Occurrence in feed and animal exposure

DDT belongs to the group of undesirable substances which are routinely analysed in the Member States within the framework of official feed controls. The aim of these monitoring programmes is to check compliance with legal limits laid down in the Annex to Directive 2002/32/EC. Unfortunately, a lot of information on the actual contamination of feeding stuffs regarding names of detected pesticides as well as their determined amount is not

¹⁹ OJ L33, 8.2.1979, p. 36

²⁰ OJ L140, 30.5.2002, p. 10

²¹ OJ L115, 4.5.1999, p. 32

²² http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp.

communicated because the Commission only requests the Member States to report their results in a condensed form as compliant or non compliant. Furthermore, it is often not specified in the condensed reports which compounds are covered by the analytical methods in the different Member States nor are the limits of detection reported. Finally, in many cases it is difficult to differentiate between numbers of individual analyses on the one hand and number of samples on the other hand. Consequently, for an evaluation of the occurrence of specific undesirable substances in feed as a prerequisite for a meaningful risk assessment, a number of subsequent queries in the Member States could be avoided if the occurrence data were to be reported in a more detailed form.

As an insecticide, DDT is predominantly applied as a spray. Therefore, vegetables and crops with large and waxy leaf surfaces grown in areas with ongoing or recent use of DDT are more likely to contain elevated DDT levels. In contrast, uptake of DDT by roots is generally low due to its low water solubility. Once uptaken by animals, DDT is metabolized mainly to DDE and DDD (see chapter 6). Hence, different contamination patterns are to be anticipated in feedingstuffs of plant and animal origin.

Recent results from EU Member States and stakeholders

In Belgium a total of 870 single and compound feed samples were collected and analysed between 2000 and 2004. All samples were negative for DDT and related compounds except three samples which contained DDT at 3, 10 and 20 µg/kg and one sample DDE at a concentration of 2 µg/kg. The analytical methods covered *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE and *p,p'*-DDD at a limit of quantification between 2 and 10 µg/kg. In connection with the Belgium dioxin/PCB case in 1999, Schepens *et al.* (2001) measured DDT and related compounds besides PCB in 750 export meat samples. While approximately 98 % of the meat samples contained levels of "DDT and its metabolites" below 20 µg/kg fat, some samples exceeded the EU maximum level of 1000 µg/kg fat. In order to identify the sources, fish flour (n = 6) and grains (n = 15) used for animal feed production were analysed. Fish flour contained DDT and DDE at concentrations of 25 ± 17 and 86 ± 71 µg/kg fat, respectively. One fish flour sample from Peru showed considerably lower levels than the other five samples originating from Northern European countries. In the 15 grain samples the concentrations for "DDT and metabolites" were found to be 0.33 ± 0.55 µg/kg product.

Estonia reported on the results of 42 feed samples, mainly grain and complete feedingstuffs which were analysed between May 2004 and March 2005 for undesirable substances. DDT could not be detected in any sample at a limit of detection of 10 µg/kg.

In Denmark 993 feed samples were analysed for undesirable substances between January 1998 and October 2004. The results for DDT are given as sum of the isomers DDT, DDE and DDD. In 98 feed samples DDT could be determined at concentrations between 1 and 132 µg/kg. Out of the 98 positive samples, 39 products were complete feedingstuffs for mink with DDT levels of 1 - 39 µg/kg and 20 products concerned complete feedingstuff mix for fish. One of the latter products contained the highest DDT level of 132 µg/kg.

In Finland 14 feed samples of plant and animal origin were recently analysed for undesirable substances. In all samples DDT (expressed as sum of DDT, DDD and DDE) was below the limit of determination. This limit was 50 µg/kg except for one sample of cod liver oil which had a limit of determination of 500 µg/kg, all based on 88 % dry matter.

Germany reported on the results of 290 feed samples of plant origin collected and analysed in 2004 for undesirable substances. None of these samples, which mainly comprised of soy bean products, citrus pulp pellets, corn pellets and palm kernel derived products, contained DDT above the limit of detection of 5 µg/kg. In 2002, some feedingstuffs (eco-wheat) were found to contain elevated levels of DDT (11 – 180 µg/kg) besides nitrofen and methoxychlor (BgVV, 2002). The source of contamination could be traced back to the storage of the eco-wheat in a warehouse which was previously used as a bulk storage for pesticides. This incident demonstrates the need for proper storage conditions for feedingstuffs in order to avoid possible secondary contamination.

Norway reported on the results of DDT occurrence in 127 feed commodities analysed between 2000 and 2004. Analyses covered *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD, each with a limit of detection below 1 µg/kg. Expressed as sum of DDT, the levels in 98 compound feedingstuffs, 10 fish meals, 2 fish oils, 6 rape seed oils, 2 fish silages and 9 vegetable feed materials (soy bean meal, corn gluten meal etc) were found to be 5 - 65, 4 - 17, 27 - 36, 3 - 13, 11 - 13 and < 0.1 µg/kg, respectively. Another 51 feed samples were analysed in 2005. While in 18 fish feed samples the sum of DDT ranged between 7.1 and 52 µg/kg (average: 24), the corresponding levels amounted to 2.3 - 15, 27 - 201 and 1.6 - 12 µg/kg in fish meal (n = 18; average 7.8 µg/kg), fish oil (n = 10; average: 95 µg/kg) and vegetable oil (n = 12; average: 5.4 µg/kg). The concentration of *p,p'*-DDE relative to the sum of DDT in these samples is more than 0.5 in complete fish feed, fish meal and fish oil, but less than 0.25 in vegetable oil. In contrast, the concentration of *p,p'*-DDT relative to the sum of DDT was only about 0.1 in fish-derived products, whilst in vegetable oils it was approximately 1/3. Julshamn *et al.* (2002), measured DDT and metabolites in farmed salmon and the corresponding fish feed at three sampling times between 1995 and 2001. The results are given as sum of DDT. The concentrations of sum of DDT in fish feed for the three sampling times ranged between 5 and 68 µg/kg product. Values in salmon fillets were typically around 20 µg/kg wet weight, and the maximum concentration was 56 µg/kg wet weight.

In 2004, detailed analyses of DDT and related compounds in 18 fish meal samples were performed in the Czech Republic. In all samples *p,p'*-DDE revealed the highest levels ranging between <0.5 and 11.9 µg/kg. Concentrations of *p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD were found to be < 0.5 - 1.7, < 0.5 - 4.0, < 0.5, < 0.5 - 4.6 and < 0.5 - 0.82 µg/kg, respectively.

Iceland reported on the results of 33 fish meal and 21 fish oil samples analysed in 2003/2004 for organochlorine pesticides. The levels of DDT expressed as the sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD ranged from 2.1 - 30.8 and 20.4 - 370 µg/kg, respectively. In all cases *p,p'*-DDE amounted to more than 50 % to the sum of DDT.

The highest concentrations were found in three blue whiting samples which were caught during or just after spawning.

Recent data on 16 fish feed samples for salmonides provided by the European Feed Manufacturers Federation showed concentrations for *p,p'*-DDE between < 5 and 15 µg/kg. Other DDT isomers or breakdown products were not detected each at a limit of detection of 5 µg/kg. Analyses of 9 further fish feed samples for salmonides with a more sensitive method of analysis resulted in DDT levels (expressed as sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) between 6.7 and 35 µg/kg. In all of these latter samples *p,p'*-DDE amounted to more than 50 % to the sum of DDT.

In conclusion, the data on the occurrence of DDT in feedingstuffs indicate that fish-derived products are generally more highly contaminated than feed materials of plant origin with the possible exception of those commodities that originate from areas with ongoing use of DDT or products that have been secondarily contaminated. Moreover, *p,p'*-DDE represents the predominant constituent in feed samples of animal origin, normally amounting to more than 50 % of the sum of DDT. In contrast, feed commodities of plant origin are mainly dominated by the parent DDT compounds.

Geographical variation of contamination

As POPs are environmental contaminants with a strong potential for bioaccumulation, these chemicals are expected to be present in farm animals and food products of animal origin which represent the predominant source for human exposure (Weiss *et al.*, 2005). Analysis of butter samples has proven to be an effective means of assessing the global contamination of persistent organic pollutants. Butter may be seen as a representative sample type for several dairy products, because it is a homogeneous, fat-rich matrix usually composed of milk fat from numerous farms. The results of the analyses of 64 butter samples from 37 countries for DDTs expressed as sum of *o,p'* - *p,p'*-DDE, DDD and DDT are depicted in Figure 3 (Weiss *et al.*, 2005). In general, the data indicate a wide range of DDT levels in butter from different countries. Butter samples in some countries from Eastern Europe had elevated concentrations of DDT and its metabolites, with a particular high concentration in Ukraine, followed by samples from Bulgaria, Russia, Hungary, Poland and Slovenia. Keeping in mind that for most countries only one sample was analyzed, these results give some indication of those countries where elevated DDT pollution exists (with great variation, see data from Russia) and consequently also feed materials produced in the respective area might contain increased DDT levels. The data from Russia also indicate that the high levels found may be an exception and not the normal.

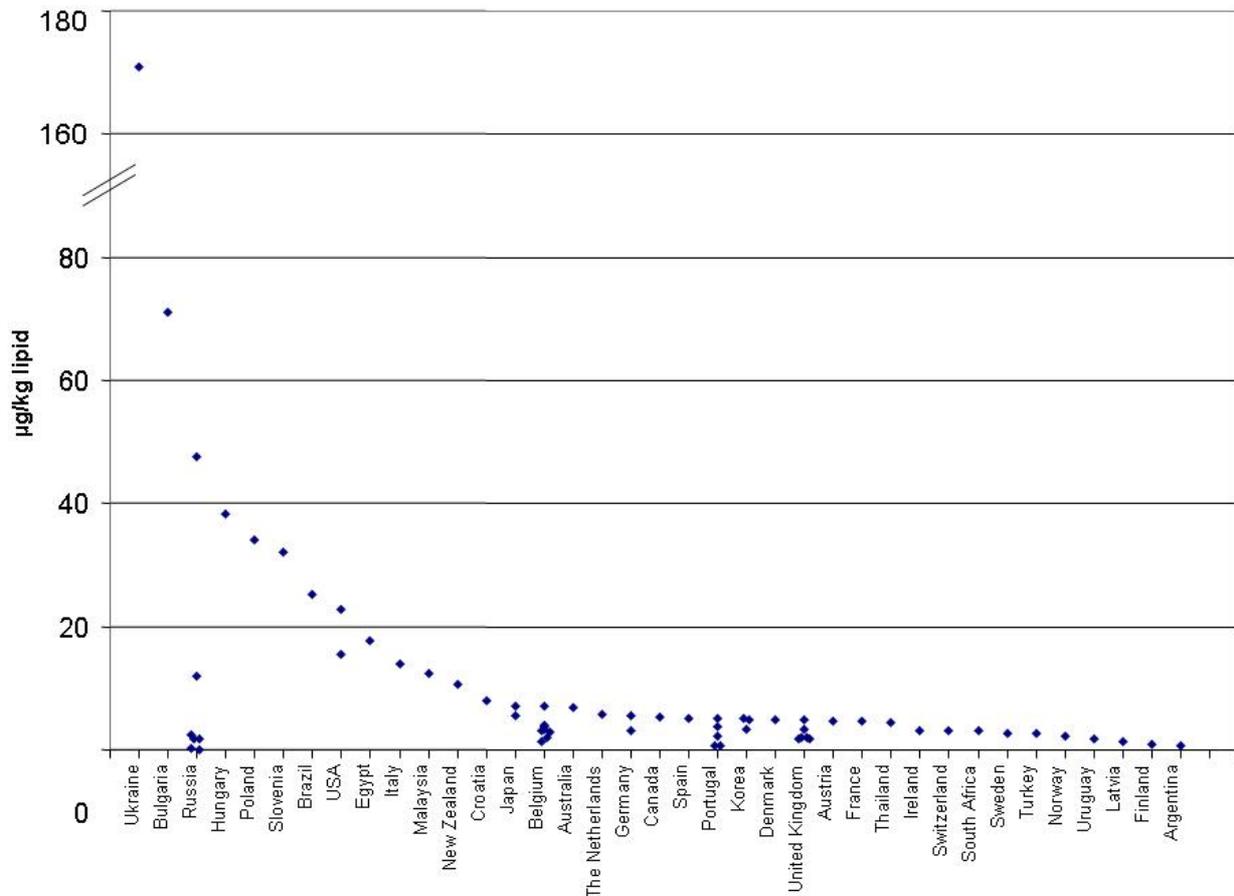


Figure 3. Concentrations (µg/kg lipid) of DDT (sum of *o,p'* - *p,p'*-DDE, DDD and DDT) in butter samples from various countries. Each dot is representing a single butter sample (data from Weiss *et al.*, 2005). The countries are arranged in order of the highest concentrations.

5. Adverse effects on fish, livestock and pets, and exposure-response relationship

5.1. Introduction

In a number of studies, particular the old ones, it was not specified whether technical grade or purified DDT was used.

Fish and terrestrial animals may be exposed to DDT and related compounds through contaminated diet. The compounds are more toxic when administered in oil solutions. In addition, fish may be exposed through the water and sediments, and livestock through dermal application. DDT has very low acute toxicity and single or repeated dermal application of DDT (usually 1.5 % concentration, vehicle not given) in toxicological studies on calves, steers, lambs, adult sheep, kids, goats, hogs and horses did not produce intoxications (Radeleff *et al.*, 1955). Howell *et al.* (1947) sprayed dairy cows with 5.0 % suspensions of DDT daily for 14 days without producing symptoms of intoxication.

The sensitivity to DDT exposure varies with species, strain, age, gender, health status and fat depot. Lean animals are more susceptible to intoxications than fat animals, since the insecticide is deposited in fat.

Acute intoxication is expressed through stimulations of the central nervous system. The symptoms vary considerably but are predominantly neuromuscular. The onset of clinical signs depends on the dose applied or ingested (Humphreys, 1988).

The signs of chronic toxicity are principally similar to those of acute intoxication but use to develop more gradually and tremors, convulsions, and depression may occur for weeks. Liver enlargement and necrosis are also common effects (Humphreys, 1988).

5.2. Fish

In general, DDT is highly toxic to fish exposed via water. The 96 hours LC_{50} range from 1.5 to 56 $\mu\text{g/L}$ (WHO, 1989). The corresponding toxicity of DDD and DDE has been less studied than that of DDT but available data show that these compounds are less toxic than DDT.

The acute effect of oral treatment of a single dose of 0.5, 1 or 5 mg/kg b.w. DDT on fitness of cod was determined (Olofsson and Lindahl, 1979). All fish treated with 5 mg/kg b.w. died within 3 - 4 days. Cod fed 1 mg/kg b.w. showed significantly reduced fitness as measured by a decrease in the capacity for compensate its posture to cope with a rotating tube in which it was swimming. The lowest dose did not show significantly changed performance.

Behavior of flounders orally treated with *p,p'*-DDT in gelatin capsules three times within one week (total doses 2.5 or 12.5 mg/kg b.w.) showed in the following week hyperactivity and changed diurnal rhythm in comparison with the control group (Bengtsson and Larsson, 1981).

Buhler *et al.* (1969) fed fingerling chinook and coho salmon with different concentrations of DDT for up to 95 days. Technical DDT (77.2 % *p,p'*-isomer) and purified *p,p'*-isomer were used and the fish were fed until they stopped eating the slowly sinking feed. Purified *p,p'*-DDT was slightly more toxic to these two species than technical DDT. The highest concentration that did not increase the cumulative mortality during 95 days exposure was 6.25 mg/kg diet. The extrapolated 90-day LD_{50} for DDT (no distinction was made between technical and purified *p,p'*-DDT) in the juvenile chinook and coho salmon were 0.028 and 0.064 mg/kg b.w. per day, respectively. The liver size decreased and the carcass lipid content increased with prolonged feeding with DDT. In addition, in coho salmon, a severe surface ulceration of the nose region and a degeneration of the distal convoluted tubule in the kidney were observed. The size of the fish greatly influenced the toxicity, as smaller juvenile coho salmon were more susceptible than larger ones (Buhler and Shanks, 1970). A no observed adverse effect level of 6.25 mg/kg diet (corresponding to a range of 0.1 - 0.3 mg/kg b.w. per day) can be derived from the study.

Juvenile rainbow trouts were fed ^{14}C -*p,p'*-DDT at 0.2 or 1.0 mg/kg b.w. per week for 140 days (Macek *et al.*, 1970). The growth of the fish was not affected but the lipid content of the

carcass was increased at the highest dose level compared with the lower dosage and the control groups.

Juvenile Atlantic menhaden were fed ^{14}C -*p,p'*-DDT at up to 93 mg/kg diet (1.9 - 2.8 mg/kg b.w. per day) for 48 days to study accumulation, retention and growth of the fish (Warlen *et al.*, 1977). No effect on growth of the menhaden was seen.

Fathead minnows were exposed to DDT at 46 mg/kg diet for 266 days through a reproductive period of their life cycle (Jarvinen *et al.*, 1977). The exposure reduced the survival of the test fish, but did not reduce the hatchability or the survival of the larvae.

5.3. Ruminant

Cattle

A single oral dose of DDT at 100 mg/kg b.w. to eight calves (1 - 2 weeks old) caused no clinical effect, while a single dose of 250 mg/kg b.w. to one calf caused mild symptoms and a dose of 500 mg/kg b.w. to another calf caused severe neurological symptoms with conspicuous tremors and seizures (Radeleff *et al.*, 1955). The acute potency of DDD in calves was found to be similar to that of DDT.

A single oral dose of 100 mg *p,p'*-DDT/kg b.w. to three-month old calves produced no clinical effect and also failed to induce hepatic aminopyridine N-demethylase (Ford *et al.*, 1976).

A single oral dose of DDT at 500 mg/kg b.w. to a steer produced neurological symptoms, but the animal recovered on the sixth day after treatment (Welch, 1948).

Three dairy cows were fed 100 mg/kg b.w of DDT daily for 6 days. For two of the cows the dose was increased to 150 mg/kg b.w. daily for 6 days and then further increased to 200 mg/kg b.w. for another 6 days. The third cow received the 100 mg/kg dose for the whole period (Orr and Mott, 1945). All three cows showed neurological symptoms during the first week, but all survived the treatment. A fourth cow that was given 200 mg/kg b.w. of DDT daily for 6 days was severely affected, but survived.

Radeleff *et al.* (1955) fed a lactating cow DDT at 100 mg/kg b.w. daily for 23 days. At the first 16 days no evidence of toxic symptoms was observed, but then the cow started to loose weight rapidly, and slight evidence of excitation was noted on the last three days of administration. No gross lesions were observed upon autopsy.

Dairy cows and heifers were fed technical grade DDT at 30, 300 or 600 mg/kg total diet (on average 0.85, 8.7 or 17 mg/kg b.w. per day) from the 90th to the 30th day before expected parturition in order to study the residue concentrations in milk, subcutaneous fat samples, blood and urine (Laben *et al.*, 1965). Toxic symptoms and reproductive effects were also examined. No adverse effects were found even at the highest dose tested (600 mg/kg diet, corresponding to 17 mg/kg b.w. per day).

Sheep

Adult sheep were given 500, 1000, 1500 or 2000 mg/kg b.w. of DDT as a single oral dose (one animal in each dose group) (Orr and Mott, 1945). Slight neurological symptoms were observed only at the dose of 2000 mg/kg b.w. Welch (1948) dosed adult sheep with a single oral dose of DDT at 500, 1000 or 2000 mg/kg b.w. Slight neurological symptoms with recovery after 24 hours were found at the lowest dose. Muscular tremors and incoordination with recovery after 48 hours were observed at the middle dose. Severe neurological symptoms lasting for 5 days and recovery on the 9th day were found at the highest dose.

Radeleff *et al.* (1955) found that three out of four adult sheep treated with a single oral DDT dose at 500 mg/kg b.w., were severely clinically affected, however the fourth individual was unaffected. A single dose of 1000 mg DDT/kg b.w. caused severe clinical effects in the two sheep treated. A single dose of DDD at 1000 mg/kg b.w. did not produce any clinical effects in five adult sheep treated.

Ford *et al.* (1976) found no clinical effects but induced hepatic aminopyridine N-demethylase which fell to pre-dosing levels by three weeks, in 6 to 9-month old sheep after a single oral dose of 100 mg *p,p'*-DDT/kg b.w.

Orr and Mott (1945) fed sheep with DDT at 100 mg/kg b.w. daily for six days, followed by 150 mg/kg b.w. for six days, and 200 mg/kg b.w. for a third six-day period. These sheep showed no evidence of intoxication other than a loss in weight. Welch (1948) found severe neurological symptoms in sheep treated with DDT at 100 mg/kg b.w. for 10 days.

Ewes aged 1 ½ years were fed *o,p'*-DDT at 10 mg/kg diet (approximately 0.3 mg/kg b.w. per day) for periods of 2 to 9 months (Wrenn *et al.*, 1971). The reproductive function was studied and no effects were found on estrogen-sensitive uterine and ovarian factors.

Yearling ewes were fed a ration containing technical DDT at 250 mg/kg (approximately 7 mg/kg b.w. per day) for 10 or 16 weeks (Cecil *et al.*, 1975). No differences were found in feed consumption, body weight, liver weight, or uterine weight, water and glycogen. Examination of the ovaries indicated that all animals were cycling. The treatment increased liver microsomal enzyme activity (aniline hydroxylase and N-demethylase).

Goat

In goats, a single oral dose of 500 or 1000 mg/kg b.w. produced neurological symptoms which disappeared within a week (Spicer *et al.*, 1947). Of four goats given a daily dose of 1000 mg/kg b.w., two were sacrificed in a moribund state after six doses, one died after nine doses and the fourth after 11 days (Spicer *et al.*, 1947). The authors concluded that the susceptibility of goats to DDT intoxication was at least in part dependent on the amount of body fat as the goats with the highest fat content survived the highest number of doses.

A nursing goat was given DDT at 50 - 100 mg/kg b.w. per day five days a week for 8 weeks (Spicer *et al.*, 1947). The goat showed some nervous tension during the treatment but the kid did not show indication of DDT intoxication.

5.4. Horse

One horse was given DDT orally at 200 mg/kg b.w. daily for six days and the only symptom observed was weight loss (Orr and Mott, 1945). A second horse was given orally 100 mg/kg b.w. daily for 6 days and then 150 mg/kg b.w. daily for 6 days and 200 mg/kg b.w. for the third six day period. Also this horse lost weight, but showed no other evidence of intoxication.

Two ponies were given a single oral dose of *p,p'*-DDT at 100 mg/kg b.w. The treatment produced no clinical effects, but induced hepatic aminopyridine N-demethylase enzyme activity which fell to pre-dosing levels after four weeks (Ford *et al.*, 1976).

5.5. Pig

No toxicological studies on pigs could be identified.

5.6. Domestic bird

DDT compounds have a low to moderate toxicity in birds. The mean lethal concentrations in feed after five days exposure of DDT in young Bobwhite quail, Japanese quail, mallard duck and pheasant were found to be 610, 570, 1870 and 310 mg/kg, respectively (Hill *et al.*, 1975). For DDE the corresponding mean lethal concentrations in feed in the same species were 830, 1360, 3570 and 830 mg/kg, respectively. For DDD these corresponding figures were 2180, 3170, 4810 and 450 mg/kg, respectively. In longer term studies, birds vary greatly between species in their sensitivity to DDT and its metabolites. Gallinaceous birds are relatively insensitive to the compounds but predatory birds in contrast are very sensitive to them.

Chicken, cock and laying hen

From seven days of age, chicks were fed DDT at concentrations of 2500 or 5000 mg/kg in their feed (Rosenberg and Adler, 1950). Those fed the higher concentration displayed marked tremors and hyperexcitability before death between 36 and 114 hours. Chicks fed the lower concentration died within a period of between 54 and 162 hours.

Four weeks old cockerels were fed technical grade DDT at 12.5, 25 or 37.5 mg/kg b.w. per day (approximately 125, 250 and 375 mg/kg diet) for 24 weeks in order to study testicular pathology (Balasubramaniam and Sundararaj, 1993). Reduced size and pathological changes in testes were found at all DDT dosage levels in comparison with the controls. The effects on the testis were dose dependent.

Male chicks above five weeks of age were fed technical grade DDT, *p,p'*-DDT or *o,p'*-DDD at 100 mg/kg diet for 10 - 30 days (approximately 10 mg/kg b.w. per day) to study adrenal cortical function (Srebocan and Pompe, 1970). It was found that technical grade DDT and *o,p'*-DDD inhibited corticosterone synthesis whereas *p,p'*-DDT did not. Technical grade DDT

at doses of 5, 50 or 500 mg/kg diet was also fed over 57 days to male chicks of above five weeks of age in order to study corticosterone levels in the adrenals and the liver glycogen (Srebocan *et al.*, 1970). At all dosage levels, adrenal corticosterone and liver glycogen levels were reduced. A LOAEL for technical DDT of approximately 0.5 mg/kg b.w. per day (5 mg/kg diet) based on reduced adrenal corticosterone and liver glycogen levels can be derived from the study.

Chicken were fed *p,p'*-DDT at 10, 30 or 50 mg/kg b.w. in gelatin capsules on alternate days from age 8 - 38 days (Radhakrishnan *et al.*, 1972). Six days following the first DDT dosage the birds were orally exposed to embryonated eggs of the nematode *Heterakis gallinarum* which transmit the protozoan *Histomonas meleagridis*. Almost all of the birds exposed to *p,p'*-DDT showed pathognomonic cecal lesions of histomoniasis. When birds not exposed to DDT were given the nematode eggs, no lesions were found. It can be derived from the study that 5 mg/kg b.w. per day (corresponding to 50 mg/kg diet) and higher doses of *p,p'*-DDT seemed to depress the immune system.

Four weeks old male broilers were fed technical DDT at different dietary levels up to 2700 mg/kg feed for up to four weeks. The main purpose was to determine the effects on antibody production following intravenous exposure to inactivated *Salmonella pullorum* or purified bovine serum albumin (Latimer and Siegel, 1974). Upon immunisation no consistent DDT effects were observed on antibody titers. Clinical symptoms were not reported for birds fed 300 mg/kg diet or lower levels. All of the birds fed with 900 mg DDT/kg diet exhibited moderate signs of intoxication such as tail tremors, stumbling gait and reduced feed intake before the end of the experiment. The birds fed with the 2700 mg/kg diet exhibited overt ataxia and died within 12 days of start of feeding. A NOAEL of approximately 30 mg/kg b.w. per day (300 mg/kg diet) for clinical symptoms.

Mature White Leghorn males were fed *p,p'*-DDT at 100 mg/kg diet for 32 weeks in order to determine the effect on reproductive performance (Arscott *et al.*, 1972). No effects were found on semen volume, packed cell volume, fertility, hatchability or testis weight. The body weight of the exposed birds was lower than that of the controls. Instances of tremors were noted at 15 weeks. In a parallel study with *p,p'*-DDE, the males were fed 100 mg/kg diet for 16 weeks and then 200 mg/kg for the following 16 weeks. No effects were found on reproductive performance or body weight, but tremors were noted in one bird at 31 weeks. It can be derived from the study that *p,p'*-DDT of 100 mg/kg diet (approximately 6 mg/kg b.w. per day) reduced body weight and induced instances of tremor. *p,p'*-DDE at 100 - 200 mg/kg diet (approximately 6 - 12 mg/kg b.w. per day) induced tremor in one bird.

Laying hen

In a 12 weeks study on general and reproductive effects on laying hens fed a diet containing DDT at levels of 310, 620, 1250 or 2500 mg/kg (Rubin *et al.*, 1947) no clinical signs of toxicity or effects on body weight were observed in hens at the two lowest dosage levels. However, egg production fell in hens given the lowest level diet, and in those fed the second lowest concentration in addition reduced hatchability was observed. Birds fed with the two highest levels of DDT exhibited strong toxic symptoms including molting, marked tremors,

poor coordination, twisting of the neck with head held down, a desire to rest on keel or side, emaciation and death. A LOAEL of approximately 20 mg/kg b.w. per day (310 mg/kg diet) for reduced egg production can be derived from the study.

To study its effect on egg production and egg shell thickness, Smith *et al.* (1970) fed laying hens with a diet containing DDT at amounts of 0, 1, 2.5, 5, 7.5 and 10 mg/kg diet for two months. Reduced egg production and egg shell thickness were found in the group fed 10 mg/kg diet. A NOAEL of approximately 0.5 mg/kg b.w. per day (7.5 mg/kg diet) for reduced egg production and shell thickness) can be derived from the study.

The effect of low dosage levels of technical DDT on fertility and hatchability were determined when White Leghorn pullets were fed the pesticide at amounts of 0.1, 1.0 and 10 mg/kg in the diet for 10 weeks (Sauter and Steele, 1972). All dose levels were found to result in increased embryonic mortality, and reduced hatchability, egg production and shell thickness. The Panel noted that this study showed effects at doses much lower than usually observed for this species.

Davison and Sell (1972) reported no effect on egg production or egg shell thickness when *p,p'*-DDT was given at concentrations of 100 or 200 mg/kg diet to White Leghorns pullets for 12 weeks (approximately 6 and 12 mg/kg b.w. per day).

White Leghorn pullets were fed diets containing *p,p'*-DDT, *o,p'*-DDT or *p,p'*-DDE at 5, 25 or 50 mg/kg for 28 weeks (approximately 0.3, 1.5 and 3 mg/kg b.w. per day) to study reproductive performance and egg shell characteristics (Cecil *et al.*, 1972; Lillie *et al.*, 1972). All isomers, particularly DDE, were found to cause increased body weight. Reproductive performance and egg shell quality were not impaired. Increasing the levels of the compounds (50, 150 and 300 mg/kg) for the next 12 weeks did however reduce the egg production. Thus, *p,p'*-DDT, *o,p'*-DDT or *p,p'*-DDE at 5 mg/kg diet for 28 weeks and then 50 mg/kg diet for the next 12 weeks reduced egg production, but 50 mg/kg diet of either of these compounds for 28 weeks did not.

White Leghorn pullets or yearling hens were fed technical grade DDT or a similar combination of DDT isomers or *p,p'*-DDT at 10 or 50 mg/kg in feed rations containing normal or low calcium content for up to 40 weeks to study the effects on reproductive performance (Cecil *et al.*, 1973; Lillie *et al.*, 1973). The predominant effect observed was reduced thickness of eggs from yearling hens fed the normal calcium level at both dosage levels of technical grade DDT. A LOAEL of approximately 0.6 mg/kg b.w. per day (10 mg/kg diet) for reduced egg shell thickness can be derived from the study.

From a 10 weeks study of White Leghorn hens fed a commercial DDT product at 20 or 100 mg/kg in a diet that contained adequate or reduced calcium levels, no significant effects on egg production, hatchability of fertile eggs or breaking strength were reported (Scott *et al.*, 1975).

Diets containing technical grade DDT at 300, 600 or 1200 mg/kg were fed to White Leghorn hens for three consecutive 28 days trial periods to study its effect on mortality, egg production

and egg shell quality (Britton, 1975a). There were no significant effects compared to controls at the lowest dosage level. The middle dosage level resulted in reduced shell thickness in all three trial periods, and reduced egg production and egg weight during the third trial period. The highest dosage level resulted in reduced egg shell thickness in all the trial periods, decreased egg weight in the second and third periods, and tremors, increased mortality and decreased egg production the third trial period. Britton (1975b) showed that all concentrations of DDT given to the laying hens increased the ability of their liver microsomal enzymes to metabolise estrogen *in vitro*. Maximal effect on metabolism (about a 2-fold increase) was reached after 14, 21 and 35 days, respectively for samples from birds fed 1200, 600 and 300 mg/kg diet. A NOAEL of approximately 18 mg/kg b.w. per day (300 mg/kg diet) for egg production and shell quality but at this dosage level induction of estrogen metabolising enzymes is still present.

In one study with technical DDT reduced egg production and reproductive effects were found at 0.006 mg/kg b.w. per day (0.1 mg/kg diet) (Sauter and Steele, 1972). However, this study was performed with technical DDT and there are several other studies on the same species with *p,p'*-DDT showing much less susceptibility. It cannot be excluded that this discrepancy can be due to contamination of the technical DDT with more toxic compounds, therefore this study will not be further considered in this opinion.

Summary for chickens, cocks and laying hens

In chicken, adrenal cortical function and liver glycogen level were affected at a level of technical DDT at approximately 0.5 mg/kg b.w. per day (5 mg/kg diet). Clinical symptoms were found at higher concentrations of technical DDT (NOAEL of approximately 30 mg/kg b.w. per day (300 mg/kg diet)). Depressed immune system seemed to occur at 5 mg/kg b.w. per day (50 mg/kg diet) and higher doses in a study with *p,p'*-DDT.

In mature cocks *p,p'*-DDT at 6 mg/kg b.w. per day (100 mg/kg diet) reduced body weight and induced instances of tremor. *p,p'*-DDE at 6 - 12 mg/kg b.w. per day (100 - 200 mg/kg diet) induced tremor in one bird.

For laying hens, reduced egg production and reduced egg shell thickness seem to be the critical clinical effect with NOAELs between 0.5 and 18 mg/kg b.w. per day (7.5 - 300 mg/kg diet). There is not enough data to conclude on differences between DDT-formulations.

Turkey

Both male and female six-week-old turkeys were fed diets containing *o,p'*- or *p,p'*-DDT at 265 mg/kg (approximately 16 mg/kg b.w per kg) for up to 15 weeks (Simpson *et al.*, 1972). Selected clinical parameters were determined to include blood pressure, plasma calcium and cholesterol levels. In addition, gross and micro-pathological examinations were performed. No effects were found.

Quail

Immature (25 day-old) and mature (50 day-old) Japanese quail were fed technical DDT (0, 100, 300, 500 or 700 mg/kg diet) until they were 80 days old to study feed consumption, body weight gain, egg production, fertility, and effect of starvation (Cross *et al.*, 1962). The birds

fed 100 and 300 mg/kg diet showed reduced survival compared to control birds when they were starved after termination of the DDT exposure, and mature birds at 300 mg/kg showed also increased mortality before starvation. The higher DDT concentrations (500 and 700 mg/kg diet) reduced body weight gain, food consumption and egg production, and produced 100 percent mortality within the exposure period. A LOAEL of approximately 6 mg/kg b.w. per day (100 mg/kg diet) based on reduced survival after starvation can be derived from the study.

Mature Japanese quail were fed a diet containing *p,p'*-DDT at 100, 200 or 400 mg/kg for up to 60 days to determine its effect on mortality, egg production, fertility and hatchability (Smith *et al.*, 1969). No effects were found at 100 or 200 mg/kg diet in comparison to unexposed controls. However, quail exposed for 30 days to the 400 mg/kg diet showed a marked decline in fertility and 50 % of the adult birds died. One-day old chicks of the birds fed on the 400 mg/kg diet for 30 days exhibited ataxia and spasms. A NOAEL of approximately 12 mg/kg b.w. per day (200 mg/kg diet) for mortality and reproductive performance can be derived from the study.

When young Japanese quail were fed 100 mg/kg of *p,p'*-DDT or *o,p'*-DDT in a diet (6 mg/kg b.w. per day) with low content of calcium in a 45 days experiment, Bitman *et al.* (1969) found that exposure to either compounds produced a lag in egg production for about 3 weeks, reduced shell calcium and more broken eggs compared to control quail fed no DDT. In addition, the eggs produced by birds fed on *p,p'*-DDT were smaller than those produced by birds on *o,p'*-DDT or control diet.

The effects on egg production and egg shell quality of young Japanese quail fed a diet containing *p,p'*-DDT or *p,p'*-DDE at 100 mg/kg diet and adequate calcium content were studied by Cecil *et al.* (1971) over a period of 74 days (6 mg/kg b.w. per day). A 3-weeks lag in egg production was found for both exposed groups in comparison to controls and there was a tendency, although it was not significant, towards more broken eggs in the exposed groups. The quail fed on the *p,p'*-DDT diet, were found to have significantly less shell calcium in their eggs.

Japanese young female quail were fed DDT at 100 mg/kg, DDE at 150 mg/kg or DDA at 200 mg/kg diets for 120 days (approximately 6, 9 and 12 mg/kg b.w. per day, respectively) to study reproductive efficiency and thyroid effects (Richert and Prahlad, 1972). Decreased reproductive efficiency and increased thyroid follicular size were found for both the DDT and DDE trials.

The impact of *p,p'*-DDT at a dosage level of 15 mg/kg diet (approximately 0.9 mg/kg b.w. per day) on egg production, fertility and number of abnormal eggs produced was studied by Carnio and McQueen (1973) over three generations of Japanese quail. In this study, the differences between quail exposed to DDT and control quail became more pronounced in subsequent generations, showing significantly reduced egg production and fertility, and an increased number of abnormal eggs in second and third generation birds.

A further study followed Japanese quail, fed either *p,p'*-DDE at 100 mg/kg diet for eight 28-day periods, or *p,p'*-DDE at 100 or 300 mg/kg diet or *p,p'*-DDT at 100 mg/kg diet for six 28 day periods using feed with reduced or adequate calcium level (Robson *et al.*, 1976). In diets containing 100 mg/kg DDT (approximately 6 mg/kg b.w.), no effects were observed. In diets containing 100 mg/kg DDE no effects were observed in egg shell thickness, cracked eggs, egg production, feed consumption, egg weight, fertility or hatchability. DDE at 100 mg/kg diet reduced the body weight of males, and DDE at 300 mg/kg diet also reduced female body weight and increased the mortality. DDE at 300 mg/kg with low calcium decreased fertility.

The effects of feeding DDT at dietary concentrations of 5 and 50 mg/kg on growth, viability and reproduction of offspring were examined in a four generation study of Japanese quail (Shellenberger, 1978). At 50 mg/kg diet there was evidence of a marginal decrease in egg hatchability in the second generation and this could relate to the slight decrease in fertility that was observed. A NOAEL of approximately 0.3 mg/kg b.w. per day (5 mg/kg diet) based on reduced fertility and hatchability can be derived from the study.

The effect of ingestion of technical grade DDT (50 or 250 mg/kg diet for nine weeks; approximately 3 and 15 mg/kg b.w. per day), *p,p'*-DDT (250 mg/kg diet for five weeks; approximately 15 mg/kg b.w. per day) or *p,p'*-DDE (300 mg/kg diet for five weeks; approximately 18 mg/kg b.w. per day) might have on the weight and histological and histochemical structure of the adrenals of Japanese quail chicks was examined by Biessmann and Von Faber (1981). The study found that adrenal weight and the percentage of adrenal cortical tissue tended to increase in all of the quail in all of the groupings compared with controls. However, the only statistically significant effects found were increased adrenal weight in birds on the *p,p'*-DDE diet and increased percentage of adrenal cortical tissue by those on the *p,p'*-DDT diet.

Oral administration of a low dose of *o,p'*-DDT, 0.020 mg/bird per day for 120 days was used to investigate the effects on reproductive parameters and liver histology in adult male Japanese quail (El-Gawish and Maeda, 2005). The effects observed included decreased gonado-somatic index, sperm concentration and the diameter of seminiferous tubules was reduced. Lipid infiltration of the liver was also observed in treated birds compared to controls at different periods of the experiment.

Mature Bobwhite quail were given technical grade DDT at dietary concentrations 5, 50 or 500 mg/kg for four months (approximately 0.3, 3 and 30 mg/kg b.w. per day, respectively) (Hurst *et al.*, 1974). The highest dosage level increased thyroid uptake of iodine (¹³¹I) as well as the weight of the thyroid gland and the liver. No consistent effects on body or adrenal weights were found.

The effect of DDE on avoidance responses to a moving silhouette was studied in Coturnix quail chicks (Kreitzer and Heinz, 1974). The birds were fed DDE at 50 mg/kg diet from age 7 - 15 days (approximately 5 mg/kg b.w. per day) and the response was measured daily. No effect on the behaviour of the birds was detected.

To conclude on quail, two multi generation studies are of particular importance for the risk assessment: *p,p'*-DDT at 15 mg/kg diet (approximately 0.9 mg/kg b.w. per day) over three generations of Japanese quail showed significantly reduced egg production and fertility, and an increased number of abnormal eggs in second and third generation birds. In a four generation study of Japanese quail 50 mg DDT/kg diet produced a marginal decrease in egg hatchability in the second generation. A NOAEL of approximately 0.3 mg/kg b.w. per day (5 mg/kg diet) based on reduced fertility and hatchability can be derived from the study. A conclusion on quail studies which compared effects of different DDT compounds, is that technical DDT and *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE have relatively similar potency with respect to effects on egg production.

Duck

The reproductive effects of diets containing *p,p'*-DDT (2.5, 10 or 25 - 40 mg/kg), *p,p'*-DDE (10 or 40 mg/kg) or a technical formulation of DDD (10 or 40 mg/kg) were studied in penned mallards exposed from several weeks before the onset of the first laying season until the second year of laying (Heath *et al.*, 1969). Exposure to DDT at the highest concentration led to thinning of the shell and reduced survival of the hatchlings. DDE at both concentrations severely impaired reproductive success: The eggshells were thinner than normal and cracked readily, and hatchability was reduced in whole eggs. The survival of hatchlings was not significantly affected by DDE. DDD did not cause demonstrable changes in shells, but it did impair reproductive success at both concentrations. From this study a NOAEL for *p,p'*-DDT of approximately 0.6 mg/kg b.w. per day (10 mg/kg diet), and a LOAEL for *p,p'*-DDE and DDD of approximately 0.6 mg/kg b.w. per day (10 mg/kg diet) were identified based on reproductive effects.

In a study on egg shell composition, captive mallard pairs were fed on a *p,p'*-DDE at 1, 5 or 10 mg/kg diet from late Autumn to Spring (Longcore *et al.*, 1971a). Changes in mineral composition of the egg shell were found on the 5 and 10 mg/kg diet. A NOAEL of approximately 0.06 mg/kg b.w. per day (1 mg/kg diet) based on mineral composition of the egg shell can be derived from the study.

Mallard ducks were fed DDT (75 mg/kg diet; approximately 5 mg/kg b.w. per day) to determine whether morphological alterations occur in shell glands of the ducks during the production of thin eggshells (Kolaja and Hinton, 1976). Hens were examined after 4, 6 and 7 weeks, and oedema of villous projections, pyknosis of glandular epithelium and cytoplasmic vacuolation of lining epithelium were observed together with thin eggshells.

In another study, ultrastructural alterations in the eggshell gland epithelium were examined in Mallard ducks fed DDT at 50 mg/kg diet (approximately 3 mg/kg b.w. per day) (Kolaja and Hinton, 1978). After five months of feeding, egg production was induced and the egg shell glands were examined after one month in full egg production. Oedema and endoplasmic vacuoles in cells responsible for the transport of calcium (type II epithelial cells) were found.

Captive black duck pairs were fed a *p,p'*-DDE at 10 or 30 mg/kg diet (approximately 0.6 and 1.8 mg/kg b.w. per day) from late Autumn to Spring to study egg shell quality and

reproductive success (Longcore *et al.*, 1971b). Both dose levels resulted in significant egg shell thinning and increased levels of shell cracking compared to eggs of untreated black ducks. The survival rate for embryos and ducklings was reduced.

Six-month-old white Peking ducks were fed *p,p'*-DDE at 250 mg/kg diet for 10 days (approximately 10 mg/kg b.w. per day) and the egg shell thickness was followed for the next 27 weeks (Peakall *et al.*, 1975). Eggshells from ducks fed DDE were approximately 20 % thinner than controls and the recovery of the shell thickness was slow, being less than half-way at the end of the observation period.

To conclude on duck studies, a NOAEL for *p,p'*-DDT of 0.6 mg/kg b.w. per day (10 mg/kg diet), and a LOAEL for *p,p'*-DDE and DDD of 0.6 mg/kg b.w. per day (10 mg/kg diet) based on reproductive effects can be derived. Based on mineral composition of the egg shell a NOAEL for *p,p'*-DDE of approximately 0.06 mg/kg b.w. per day (1 mg/kg diet) can be derived.

Dove

Following oral treatment of homing pigeons with *p,p'*-DDT in gelatin capsules at 18, 36 and 72 mg/kg b.w. every second day for 42 days (corresponding to 9, 18 and 36 mg/kg b.w per day; approximately 150, 300 and 600 mg/kg diet) the effects on the thyroid gland were examined (Jefferies and French, 1969). Increased weight and reduced colloid content of the thyroid accompanied by increased liver weight were found at all dosage levels. No birds died, but one of four birds exposed to the highest DDT level, developed tremors at day 30 of the trial.

Ringdoves were given feed containing 10 mg/kg of *p,p'*-DDT (approximately 0.6 mg/kg b.w. per day) from three weeks before mating until they were killed eight days after mating or until they were killed at completion of a clutch of two birds (Peakall, 1970). Of the birds killed 8 days after mating, those fed DDT showed reduced concentration of estradiol in the blood and reduced calcium level in the leg bones. The metabolic activity of the hepatic microsomal enzyme system on estradiol increased. In birds killed after completion of the clutch, there was no difference in amounts of estradiol neither in the blood nor in the calcium level in leg bones. However, egg shell weight and calcium in the eggshell were reduced in the group fed DDT, and the time from mating to laying was increased.

Adult ringdoves were fed DDE at dietary concentrations of 2, 20 or 200 mg/kg for eight weeks (Heinz *et al.*, 1980). The intermediate and highest doses depressed the levels of brain dopamine and norepinephrine, reduced feed consumption slightly and increased liver weight. A NOAEL of approximately 0.12 mg/kg b.w. per day (2 mg/kg diet) can be derived from the study.

To sum up all the bird studies, there was a large inter study variation in sensitivity to technical DDT and the related compounds. However, the variation in potency of clinical effects for the various compounds tested was in general far less. Variation in design and the birds' pre-experimental health, the composition of the experimental diet and potential impurities of the test compounds, may explain the variation of effect results.

Studies on domestic bird species have been summarised in the Annex.

5.7. Rabbit

Pregnant rabbits were orally given *p,p'*-DDT at 50 mg/kg b.w on day seven, eight and nine of gestation to study developmental effects *in utero* (Hart *et al.*, 1971). The treatment resulted in premature birth, increased numbers of resorption sites and reduced the weight of viable foetuses but there were no signs of teratogenic effect.

5.8. Dog and cat

Adult male beagle dogs were orally administered 0 or 24 mg DDT/kg b.w. in gelatine capsules five days a week for 10 months (Deichmann *et al.*, 1969). No overt clinical effects were observed. Two of six dogs treated with DDT showed focal areas of parenchymatous degeneration of hepatic tissue.

In another study, adult beagle dogs were treated orally with 0 or 50 mg/kg b.w. of *o,p'*-DDT (> 99 % purity) in gelatine capsules daily for 32 days (approximately 2500 mg/kg diet). The purpose was to investigate the effect on adrenal function and hepatic mixed function oxidase systems (Copeland and Cranmer, 1974). There was no significant effect on body weight and no overt signs of toxicity. The *o,p'*-DDT dose did not block the synthesis or release of corticosteroids by adrenal cortex but the adrenals were significantly larger and histopathological changes were found in zona fasciculata. The activity of hepatic microsomal enzyme systems was stimulated. These results differ somewhat from those of Berndt *et al.* (1967) who reported that treatment of dogs with 50 mg/kg of *o,p'*-DDT per day for seven days reduced the plasma concentrations of corticosteroids. A similar treatment of *p,p'*-DDT did not produce this kind of effect (Berndt *et al.*, 1967). However, the DDT metabolite *o,p'*-DDD was found to be a more active compound regarding adrenocortical inhibitory effect in earlier studies (Nelson and Woodard, 1949; Cueto and Brown, 1958).

No toxicological studies on cats could be identified.

6. Toxicokinetics and tissue disposition

6.1. Absorption

Absorption of DDT from the gastrointestinal tract is dependent on the dose and on the vehicle. Approximately 70 – 90 % of the administered dose is absorbed by rats after oral exposure to DDT in an oil vehicle (Keller and Yeary, 1980; Rothe *et al.*, 1957). DDT is absorbed 1.5 – 10 times more effectively in laboratory animals when given in digestible oils compared with undissolved DDT (Smith, 2001). Absorption of small doses, such as those

found in the residues of food, is virtually complete and is facilitated by the presence of fat in food.

Absorption occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation (Palin *et al.*, 1982; Pocock and Vost, 1974; Rothe *et al.*, 1957; Sieber, 1976).

No quantitative data specifically describing the absorption of DDT in domestic animals following oral exposure were found in the literature. However, the presence of DDT residues in tissues, eggs or milk of animals dietary exposed to these chlorinated compounds (Noble, 1990) indicates that gastrointestinal absorption occurred at a high extent in these species.

6.2. Distribution

DDT, DDE and DDD are lipophilic compounds. Once absorbed, they are readily distributed via the lymph and blood to all body tissues and are stored in these tissues generally in proportion of their lipid content. Following repeated doses, storage in the fat increases rapidly at first and then more gradually until a plateau is reached, in about 6 months in rats. Most species, including humans, store DDE more efficiently than DDT (Smith, 2001). In modelling DDE disposition in the pregnant rat, You *et al.* (1999) found that tissue/blood partition coefficients for adipose tissue, liver, kidney, placenta and mammary gland were 50, 7, 6, 2 and 12, respectively.

In laboratory animals and in humans DDT and corresponding metabolites have been shown to cross the placenta. In humans, a study on 90 mother/infant pairs from Mexico (Waliszewski *et al.*, 2000) found that all 90 cord blood samples had detectable levels of *p,p'*-DDE, nine had detectable levels of *o,p'*-DDE, and 44 had measurable levels of *p,p'*-DDT.

6.3. Metabolism

Several authors found that 2,2-bis-chlorophenyl acetic acid (DDA) isomers are the major urinary metabolites of *p,p'*-DDT and *o,p'*-DDT in all mammals, including humans (Smith, 2001). The conversion of DDT to DDD or to DDE are the first steps in the formation of DDA (see Figure 5). The conversion of DDD to DDA occurs primarily by hydroxylation, leading to acyl chloride DDA, which on hydrolysis, gives DDA. This acyl chloride may also be formed from DDE via an epoxidation route. Another possible intermediate in the formation of DDA from DDD is 1-chloro-2,2-bis(4-chlorophenyl) ethane (DDMU) (Gold and Brunk, 1984; Fawcett *et al.*, 1987).

In addition to the formation of DDA, several hydroxylated compounds can be formed from DDE. The major metabolite observed in the faeces of rats fed *p,p'*-DDE (Sundström *et al.*, 1975) was *m*-hydroxy-*p,p'*-DDE. *o*-Hydroxy-*p,p'*-DDE, *p*-hydroxy-*m,p'*-DDE, and *p*-hydroxy-*p'*-DDE were also present in excreta.

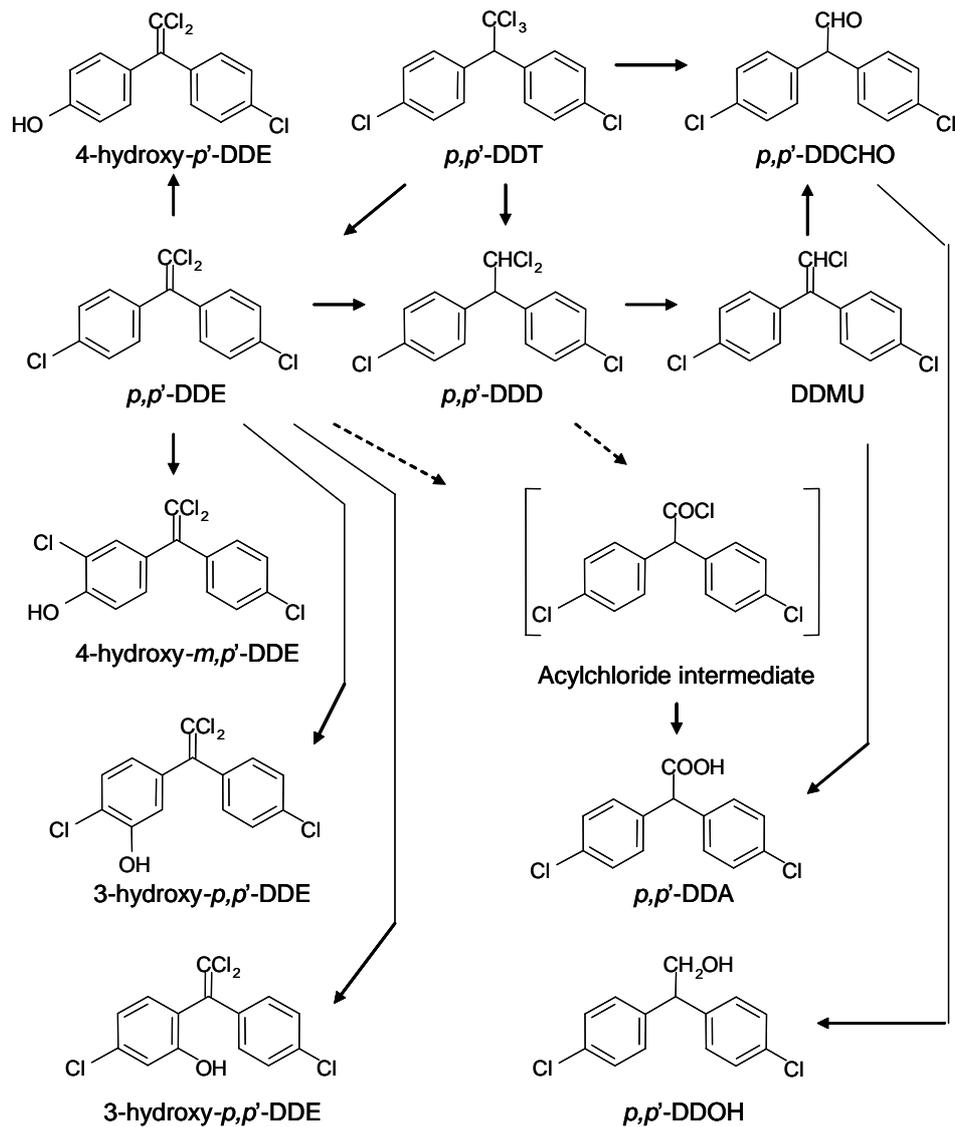
After phase I biotransformation (reactions involving oxidation, reduction and hydrolysis) many of the DDT metabolites (especially those resulting from *o,p'*-DDT) are ultimately excreted in the conjugated form, including glycine, serine and glucuronic acid conjugates (Gingell, 1975, 1976; Reif and Sinsheimer, 1975).

DDE is metabolised not only to easily excretable phenols but also to 3-methylsulfonyl-*p,p'*-DDE, suggesting an initial conjugation with glutathione of a phase I metabolite, cleavage by a C-S lyase, then methylation and further oxidation of the sulfur to the corresponding methylsulfone (Smith, 2001). 3- and 2-methylsulfonyl DDE have been found in several animal species (Bergman *et al.*, 1994) and in humans (Weistrand and Norén, 1997, Norén *et al.*, 1999, Chu *et al.*, 2003). No data on the occurrence of 2- or 3-methylsulfonyl-DDE in farm or domestic animals was found. 3-Methylsulfonyl-DDE was found in different marine mammals and arctic species and was present at the highest concentrations in liver.

The conversion of *o,p'*-DDT to *p,p'*-DDT has been described in rats (Klein *et al.*, 1965) and chicks (Abou-Donia and Menzel, 1968), but this finding was contradicted by Cranmer (1972) with respect to the rat.

Compared to *p,p'*-DDT, the more rapid excretion of *o,p'*-DDT is explained at least in part by the ring hydroxylation of the parent compound and/or its metabolite *o,p'*-DDD, observed in rats (Feil *et al.*, 1973; Reif and Sinsheimer, 1975), chickens (Feil *et al.*, 1975) and humans (Reif *et al.*, 1974). Ring hydroxylation which has not been observed with *p,p'*-DDT or *p,p'*-DDD (but has been seen with *p,p'*-DDE) occurs in all species, but with some qualitative and quantitative differences (Smith, 2001). For example three hydroxylated *o,p'*-DDEs were found in the excreta of chicken, but not in the excreta of rats.

When the metabolism of a single 100 mg oral dose of ¹⁴C-*o,p'*-DDD was studied in rat (Reif and Sinsheimer, 1975), at least 12 metabolites were detected in urine and faeces, most of them being similar to those reported for *o,p'*-DDT, in particular DDMU, DDA and various metabolites resulting from aromatic hydroxylation. A covalent binding of *o,p'*-DDD metabolites to tissue macromolecules was observed in mouse lung by Lund *et al.* (1986, 1989), probably related to a cytochrome P450-mediated activation to the acyl chloride as suggested for the toxicity of DDD to isolated rabbit Clara cells and human bronchial epithelial cells (Nichols *et al.*, 1995).



Most of the metabolites are excreted in a conjugated form.

DDOH = 2,2-bis-(4-chlorophenyl)ethanol.

DDCHO = 2,2-bis-(4-chlorophenyl)ethanal.

For other abbreviation see the text.

Figure 5. Metabolic scheme for *p,p'*-DDT in rats (adapted from Smith, 2001).

The metabolic pathway of *o,p'*-DDT is similar to *p,p'*-DDT.

6.4 Excretion

Studies with rats, mice and hamsters indicate that DDT and related metabolites are excreted primarily in faeces. Three days after a single oral dose of ^{14}C -*p,p'*-DDT (25 mg/kg b.w. by intubation) to male and female mice and hamsters, faecal excretion of radioactivity was between 24 % and 37 % of the administered dose in hamsters, and between 29 and 34 % in mice, whereas urinary excretion was between 10 and 18 % in hamsters and between 10 and 12 % in mice (Gingell and Wallcave, 1974). Total DDA (free + conjugates) was recovered from the urine of both mice (5 % dose) and hamsters (10 % dose). No significant gender difference was observed in this study. In the rat, several studies indicate that faecal excretion of DDT and

metabolites exceeds urinary excretion, irrespective of the route of administration (Hayes, 1965). Bishara and co-workers (1972) reported that of a 10 mg dose of *p,p'*-DDT orally given to rats, 2.6 % was excreted in urine and 59.4 % in faeces in 5 days. The predominance of faecal excretion over urinary elimination was also observed for *o,p'*-DDT (Feil *et al.*, 1973), *o,p'*-DDD (Reif and Sinsheimer, 1975), and *p,p'*-DDE (Mühlebach *et al.*, 1991). The bile appears to be the principal source of DDT metabolites in the faeces. Burns *et al.* (1957) found that there was an increase in urinary excretion of radioactive material following ligation of the bile duct in rats fed radiolabelled DDT. Furthermore, when the bile duct was cannulated before an intravenous injection of ¹⁴C-DDT, 65 % of the dose was recovered in bile, 2 % in urine and only 0.3 % in faeces.

Elimination half-lives for DDT range from about one month in rats and dogs to 6 - 14 months in fish (Morgan and Roan, 1974, Macek *et al.*, 1970, Warlen *et al.*, 1977). The values reported for sheep, and beef cattle, are approximately 12 - 15 weeks for DDT, 4 - 7 weeks for DDD and 30 - 32 weeks for DDE (Reynolds *et al.*, 1976; McCully *et al.*, 1966).

Milk has been reported as an important excretion route for DDT and related metabolites. In rats chronically exposed to 25 mg *p,p'*-DDT/kg diet, the residues found in milk (whole milk basis) were as follows: 32.7 mg/kg as *p,p'*-DDT, 0.7 mg/kg as *p,p'*-DDD and 2.9 mg/kg as *p,p'*-DDE (Woolley and Talens, 1971). According to these data and assuming a lactating rat consumes 60 g diet per day and produces 20 ml milk per day, the proportion of the dam's DDT intake that was recovered from her milk as parent compound and metabolites was about 50 %.

Studies in human volunteers generally show a higher percent of the dose excreted in urine (10 - 30 %) than observed in rodents. The biological half-lives for the elimination of *p,p'*-DDT and *p,p'*-DDE were estimated at approximately 4 and 6 years respectively (Norén and Meironyté, 2000). The storage loss was ranked as follows, from fastest to slowest, by Morgan and Roan (1974): *p,p'*-DDA > *p,p'*-DDD > *o,p'* DDT > *p,p'*-DDT > *p,p'*-DDE.

Whereas DDA and the corresponding conjugates are the major excretion products of DDT in mammals, including humans (Smith, 2001), and in Japanese quail (Ahmed and Walker, 1980; Fawcett *et al.*, 1987), it is only a very minor metabolite in pigeon (Sidra and Walker, 1980).

7. Carry-over and tissue concentration

7.1 Transfer into milk and eggs

In an early review, Hayes (1959) showed that cows fed DDT commonly excrete 10 % or slightly more of the total dose in their milk, and amounts slightly over 30 % have been observed. Fries *et al.* (1969) compared the transfer into milk of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE in dairy cows fed daily 25 mg of either compound for 60 days. From 40 to 60 days, corresponding to a period where the equilibrium is approached (i.e. maximum concentration in milk), 25.8 % of the *p,p'*-DDE, 7.6 % of the *p,p'*-DDD and 5.1 % of the *p,p'*-DDT were excreted in the milk.

Noble (1990) derived transfer ratios (concentration in milk or eggs relative to the concentration in the diet) from trials which have involved feeding DDT to dairy cattle and laying hens. The ratio of DDT calculated for whole milk was 0.11, whereas the values reported on a milk fat basis were within a 1.5 - 2.7 range. The latter values yielded 4.8 - 10.9 for DDE.

The transfer ratio for total DDT determined in whole eggs was between 1.3 and 1.6 (Noble, 1990). In laying hens fed diets containing 5 mg/kg feed of *p,p'*-DDT, *o,p'*-DDT or *p,p'*-DDE for 28 weeks, the concentrations in eggs reached equilibrium at 12 weeks and were approximately equal to dietary level for the *p,p'*-DDT and *p,p'*-DDE diets (Cecil *et al.*, 1972). The level of *o,p'*-DDT in eggs, however, was only 10 % of the dietary level. These data indicate the proportion of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE daily intake excreted in the egg contents were 34, 3.5, and 42 %, respectively. At the equilibrium, 75 % of the egg residues in *p,p'*-DDT exposed chicken was *p,p'*-DDT, whereas 25 % was *p,p'*-DDE.

7.2 Tissue levels and bioaccumulation

Accumulation ratios (concentration in tissues relative to the concentration in the diet, usually calculated at the plateau level) for DDT and analogs in adipose tissue of poultry and sheep have been reported (Kan, 1978; Reynolds *et al.*, 1976). These ratios, calculated on *p,p'*-DDT + *p,p'*-DDE residues in abdominal or subcutaneous fat and *p,p'*-DDT in the feed varied from 2.2 in sheep to 6 - 30 for broilers. More recently, Noble (1990) calculated, based on older published data, accumulation ratios for DDT in beef cattle, poultry and pigs. The reported values were 0.7 - 0.9, 11 - 12 and 0.4, respectively. For DDE the accumulation ratios in the fat of beef cattle were in the 11 - 26 range.

Macek *et al.* (1970) fed rainbow trout on diets containing 500 or 100 mg *p,p'*-DDT/kg feed for 24 weeks. Equilibrium (i.e. when accumulation equalled elimination) was reached after 20 weeks of exposure and at the end of the experiment fish retained 20 - 24 % of the dietary intake of DDT. Similar results were obtained by Warlen *et al.* (1977) in Atlantic menhaden, but higher values (55 and 48.2 %) were reported by Buhler *et al.* (1969) in chinook salmon fingerlings fed a diet containing 6.25 mg *p,p'*-DDT/kg feed during 65 days and in coho salmon dietary exposed to technical DDT at the same concentration during 39 days. In a more recent study Bayen *et al.* (2005) found that the Asian sea bass (*Lates calcarifer*) fed a diet containing 12 or 70 µg *p,p'*-DDT/kg feed over a 42 day period retained almost all the ingested DDT, mainly as *p,p'*-DDT (97.5 % of total residues); traces of *p,p'*-DDE and *p,p'*-DDD were also present, principally in liver and adipose tissue. According to these studies, it appears that the bioaccumulation factor in fish (concentration in organism/concentration in feed) depends on the concentration of DDT in feed and other factors, but is usually below 1.

In order to evaluate the relative importance of feed and water as sources of DDT residues to the fish, Rhead and Perkins (1984) simultaneously exposed goldfish (*Carassius auratus*), to *p,p'*-DDT from feed (³⁶Cl-*p,p'*-DDT) and water (¹⁴C-*p,p'*-DDT). The concentration of *p,p'*-DDT in feed was maintained at about 1 mg/kg while the water concentration of *p,p'*-DDT was

varied for each experiment (2, 20 and 177 ng/L). In these experiments the contribution of *p,p'*-DDT from feed to the total body residue varied over the duration of the exposure from 0 to 81 % (concentration in water about 2 ng/L) and from 3.8 to 4.8 % (concentration in water about 171 ng/L). The data suggests that *p,p'*-DDT residues in fish can be derived from both feed and water. The importance of each source is determined by its relative concentration over the period of exposure. *p,p'*-DDT taken up by goldfish was converted, slowly at first, then more rapidly to *p,p'*-DDE reaching a maximum of > 80 % from both water and food sources after about 40 days. Dietary *p,p'*-DDT was converted to *p,p'*-DDD to a greater extent (max. 15.9 %) than water-borne *p,p'*-DDT (max. 2.8 %).

Morgan and Roan (1974) gave human volunteers 5 to 20 mg technical DDT orally (77 % of *p,p'* isomer and 23 % of *o,p'* isomer) orally per day for 6 months. The steady state was not reached at the end of the experiment. The concentration of total residues measured in adipose tissue varied from 75 to 160 mg/kg, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE representing approximately 75, 10 and 15 % of this total amount, respectively.

No data was found on the bioaccumulation of 3-methylsulfonyl-DDE in animals. In humans, the ratio 3-methylsulfonyl-DDE/*p,p'*-DDE was about 1/100 in all tissues from 11 Belgian subjects collected by Chu and co-workers (2003) during a medicolegal autopsy.

8. Human dietary exposure

8.1. Dietary intake assessments

Studies on recent dietary DDT intake are scarce. This is presumably due to the long-standing ban on this compound in most countries worldwide as well as the progressive decline of DDT and its metabolites in humans as demonstrated by the analysis of human milk and serum samples over the course of time.

Between April and May 2003 the dietary intake of some persistent organic pollutants was studied in 11 German women from the Land Schleswig-Holstein at the age of 22-40 years. A total of 55 duplicate diet samples were collected. The sampling period for each participant was 5 days. The study revealed intakes for *p,p'*-DDT and *p,p'*-DDE of < 0.10 – 45.5 and 0.40 – 35.7 ng/kg b.w. per day, respectively. Mean, median and 95th percentile intake for *p,p'*-DDT and *p,p'*-DDE were found to be 2.8, 1.5 and 6.0 and 5.3, 3.8 and 14.7 ng/kg b.w. per day, respectively. The recent median dietary DDT (sum of *p,p'*-DDT and *p,p'*-DDE) intake of 5.1 ng/kg b.w. per day is approximately 23 % lower (LGASH, 2005) compared with 1997 whereas a comparable study gave a median intake of 6.27 ng/kg b.w. per day. The dietary intake of DDT and other persistent organic pollutants (POPs) was also studied in 14 German children at the age of 1.5 to 5.3 years between April and May 1995. A total of 98 duplicate diet samples were collected. The sampling period for each participant was 7 days. The median daily intake for *p,p'*-DDT and *p,p'*-DDE were 3.4 and 11.2 ng/kg b.w. per day. Minimum, 95th percentile and maximum for *p,p'*-DDT and *p,p'*-DDE were given as 0.56, 11.8 and 280 and 2.4, 28.6 and 101 ng/kg b.w. per day, respectively (Wilhelm *et al.*, 2002)

Based on a market basket study performed in 1999, the dietary intake of DDT and other organochlorine contaminants was assessed in Sweden. The estimated mean intake of DDT (calculated as the sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD) was found to be 7.1 ng/kg b.w. per day. Consumption of fish contributed 49 % to this intake followed by meat, dairy products and fats/oils with contributions of 16, 14, and 14 %, respectively. This dietary DDT exposure is considerably lower compared to 1994 where a comparable assessment revealed an approximately 4-fold higher DDT intake (Darnerud *et al.*, 2006).

Dietary exposure assessment based on national market basket studies was performed in the Czech Republic during the years 1994 to 2003 (Adamikanova *et al.*, 2003). During this period the dietary total DDT intake decreased by approximately 40 %. Based on occurrence levels in food between 1999 and 2003 and data of the Czech national consumption data base for individuals a median dietary intake (age 4 - 90 years, both genders) of 29.1 ng/kg b.w. per day was calculated for total DDT (sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD). The contribution of *p,p'*-DDE and *p,p'*-DDT to total DDT was 56 and 12 %, respectively. The intake of total DDT for highly exposed individuals, as represented by the 90th, 95th, 97,5th and 99th percentiles, were 51.8, 61.8, 71.8 and 84.6 ng/kg b.w. per day respectively.

In the 1980/1990s assessments of total DDT dietary exposure were performed in several European countries. Intake levels for Finland (1986), Netherlands (1988/1989), Poland (1997), Spain (1988-1990) and United Kingdom (1992) were given as 26, 17, 78, 23, and 2 ng/kg b.w. per day. Total diet studies performed in 1984 - 1986 and 1991 in the USA showed a decline of total DDT intake from 21.3 to 5.6 ng/kg b.w. per day (cited in WHO, 1998).

Dietary intake studies performed in Canada between 1993 and 1998 revealed an average human dietary total DDT intake (all ages, both genders) of 2.4 and 4.0 ng/kg b.w. per day (Health Canada, 2004). In Japan, the dietary intake of total DDT decreased from 71 ng/kg b.w. per day in 1980/1984 to 24 ng/kg b.w. per day in 1992/1993. Considerably higher dietary exposures to total DDT were reported for China (1990) and India (1994) with values of 342 and 321 ng/kg b.w. per day (cited in WHO, 1998).

The exposure assessments recently performed in industrialized countries indicate that food of animal origin generally contributes the highest to the dietary intake of DDT and related compounds. Consumption of fish oil may be a significant contributor to human DDT exposure. It was shown that the daily intake of total DDT derived by the consumption of cod liver oil used as a dietary supplement at manufacturer recommended doses ranged from 4 – 1240 ng per day (Storelli *et al.*, 2004).

In summary, it can be stated that the actual daily dietary intake of DDT and related compounds in those EU member states where recent exposure data are available is in the low ng/kg b.w. range and thus more than two orders of magnitude below the PTDI of 0.01 mg/kg b.w.

8.2. Levels in humans

Large differences in dietary DDT exposures between industrialized and developing countries are also reflected by the geographical variation in contamination of human milk. While many industrialized nations banned or began to restrict the use of DDT during the 1970s, at about the same time, use of DDT in developing nations was peaking. Thus, concentrations have steadily decreased in many industrialized nations, but exposure hot spots persist in countries where populations are still exposed to DDT through agricultural use, malaria control or contaminated food sources. As a consequence, populations in several countries in Africa, Asia and Latin America have considerably higher levels of DDT and related compounds in human tissue than in Europe and the United States. The global surveillance of DDT and DDE levels in human tissues and their worldwide trend in human milk are comprehensively summarized by Jaga and Dharmani (2003) and Smith (1999). The data show a wide variability between countries and in some cases also between regions within one country. Moreover, the ratio of DDT and DDE differs remarkably. While a low DDT/DDE ratio implies high environmental persistence and ongoing bioaccumulation, a high DDT/DDE ratio is an indication of ongoing exposure to DDT. (Jaga and Dharmani, 2003).

In 1998, exposure to DDT and related compounds has been studied in people (682 serum samples) of the Canary Islands, where extensive farming areas have been developed in the last decades, with greenhouses dedicated to intensive cultivation previously using DDT (banned in Spain in 1977) in huge amounts. The median concentration of total DDT was 0.37 mg/kg fat, which was similar to that found in other European countries. In a fourth of the study population the DDT concentration was higher than 0.72 mg/kg fat. A very high DDT/DDE ratio, 28 % showed values higher than 1, was observed indicating ongoing exposure to DDT. The sources of exposure are not clear (Zumbado *et al.*, 2005).

In the framework of the 3rd WHO human milk field study (Malisch *et al.*, 2004), *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE and *o,p'*-DDD were analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine), and in 11 pools from six non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA). The total DDT levels ranged from 0.12 – 2.05 mg/kg milk fat with a median value of 0.36 mg/kg milk fat. If only the European countries are considered, the total DDT levels range from 0.12 – 1.1 mg/kg milk fat with a median value of 0.19 mg/kg milk fat. While the lowest levels were found in the specimens from Norway, Luxemburg, Germany and the USA, the highest levels were determined in the samples from the Philippines, Fiji, Ukraine and Hong Kong. The contributions of *p,p'*-DDE and *p,p'*-DDT to total DDT ranged from 79 - 98 and 2 – 17 %, respectively.

8.3. Time Trend

Although human levels can still be high in areas with ongoing use of DDT, exposure studies conducted worldwide indicate that DDT concentrations in humans as reflected by the levels in

human breast milk have declined in most areas of the world (Smith 1999). This decline is most prominent for those countries which banned the use of DDT already in the 1970s, Germany, for example, has a long history regarding the monitoring of human milk for contaminants. Since the late 1970s individual human milk samples have been analysed for DDT and related compounds as well as other organochlorine pesticides and the results are reported annually. These data allow a reliable overview of the contamination of human milk in the course of time. Figure 6 shows the median levels of total DDT in more than 7000 individual human milk samples collected and analysed between 1979 and 2003 in Germany. The results for the samples from 1979 - 1983 were taken from a compilation performed by BgVV (2000), the data for the years 1984 - 2001 from Fürst (2006) and the most recent data for 2002 and 2003 from a report published by NLGA (2004). As can be clearly seen, the ban of DDT in the 1970s resulted in a substantial DDT decrease in human body burden and thus exposure to breast fed babies. Since 1979 the levels of total DDT have decreased by more than 90 %. While the average level in 1979 - 1981 ($n = 3390$) amounted to 1.83 mg/kg milk fat, the median concentration in 2003 was only 0.12 mg/kg milk fat. Actual samples from mothers with no known recent exposure show almost exclusively the metabolite *p,p'*-DDE and virtually no parent compound *p,p'*-DDT (Fürst, 2006).

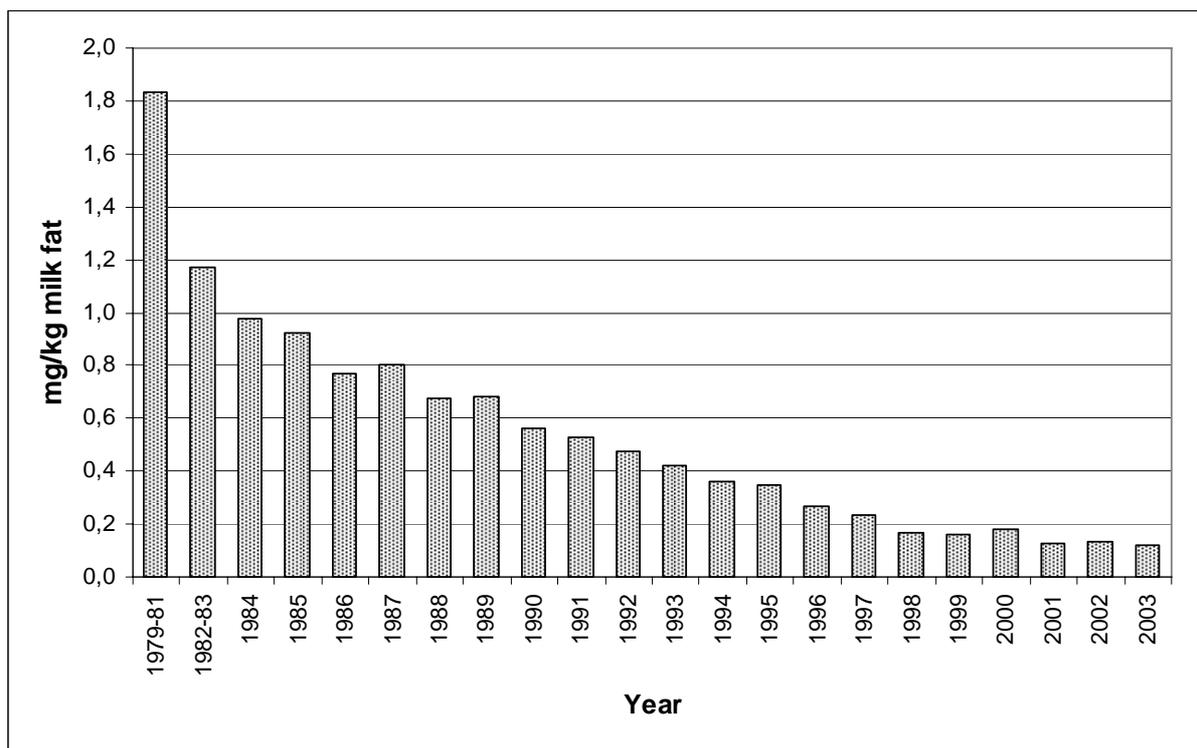


Figure 6. Temporal trend of total DDT levels in human milk samples (> 7000 individual samples from Germany) (BgVV, 2000; NLGA, 2004; Fürst, 2006).

A similar downward trend was also found for human milk from Sweden (Norén and Meironyté, 2000) and several other countries worldwide (Smith, 1999).

Assuming an average daily intake of 800 ml breast milk with a fat content of 3.5 % for an exclusively breast fed baby weighing 5 kg, a total DDT concentration of 0.19 mg/kg fat

(median value for European countries of 3rd WHO human milk field study) would result in an average daily intake of 1.1 µg/kg b.w.

CONCLUSIONS

Production, use and environmental fate

- DDT was commercially introduced as an insecticide in the 1940s. Technical DDT contains 65 – 80 % *p,p'*-DDT. Other important constituents in the technical products are *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. The latter two compounds are also the major breakdown products in biological systems. Unless otherwise stated in this opinion the terms “DDT and related compounds” and “sum of DDT” refer to *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD.
- DDT is used as an intermediate in the production of the pesticide dicofol and may occur as a major impurity in the final product. In the EU, the DDT content in dicofol is limited to 0.1 %.
- Although being banned in most countries worldwide, DDT is still used for vector control in areas with endemic malaria, and extended use was recently recommended by WHO for indoor residual spraying to control malaria.
- Because of the lipophilic properties and persistence in the environment, DDT and related compounds are bioaccumulated and biomagnified along the food chain.

General toxicological effects

- The main target organs are the nervous system and the liver. It also affects hormonal tissues, reproduction, fetal development and the immune system. DDT including *p,p'*-DDE and DDD cause tumours mainly in the liver of experimental animals and are mostly negative in genotoxicity studies. DDT is classified by IARC as possible carcinogenic to humans (group 2B). JMPR has established a PTDI of 0.01 mg/kg b.w..

Adverse effects of DDT in target animals

- DDT and related compounds have a relatively low acute toxicity in mammals and most bird species.
- DDT is highly toxic to **fish** exposed via water (LC50, 96 hours, 1.5-56 microg/L). In oral studies a no effect level of 6.25 mg/kg diet (0.1 - 0.3 mg/kg b.w. per day) can be derived based on effects in chinook and coho salmon.
- No clinical or reproductive effect was observed in **dairy cows and heifers** fed DDT during two months before expected parturition at doses up to 600 mg/kg diet (17 mg/kg b.w. per day).

- No effect on reproduction was seen in **ewes** at doses of *o,p'*-DDT at 10 mg/kg diet (0.3 mg/kg b.w. per day). **Sheep** fed technical DDT at a dose of 250 mg/kg diet (7 mg/kg b.w. per day) had increased liver microsomal enzyme activity.
- No information on long term exposure in **goats, horses and pigs** was identified.
- **Dogs** given DDT at 24 mg/kg b.w. five days a week for 10 months showed no clinical effects but histopathological changes in the liver. No information was identified for **cats**.
- In **chicken**, adrenal cortical function and liver glycogen level were decreased by technical DDT at a level of 5 mg/kg diet (0.5 mg/kg b.w. per day). Studies in **laying hens** showed reduced egg production and egg shell thickness with NOAELs between 0.5 – 18 mg/kg b.w. per day (7.5 - 300 mg/kg diet).
- In a four generation study on Japanese **quail** a NOAEL of 5 mg/kg diet (0.3 mg/kg b.w. per day) can be derived, based on reduced fertility and hatchability.
- In **ducks** a NOAEL for *p,p'*-DDT of 10 mg/kg diet (0.6 mg/kg b.w. per day) and LOAELs for *p,p'*-DDE and DDD of 10 mg/kg diet (0.6 mg/kg b.w. per day) based on reproductive effects can be derived. Based on mineral composition of the egg shell a NOAEL for *p,p'*-DDE of 1 mg/kg diet (0.06 mg/kg b.w. per day) can be derived.
- In **Ringdoves** *p,p'*-DDT at a dietary level of 10 mg/kg (0.6 mg/kg b.w. per day) reduced blood concentration of estradiol and bone and egg shell calcium levels. Brain transmitter levels were reduced when fed DDE and a NOAEL of 2 mg/kg diet (0.12 mg/kg b.w. per day) can be derived.

Contamination of feed

- Recent occurrence data indicate that feed materials of animal origin, especially fish derived products, are in general more contaminated than feed materials of plant origin.
- In feed samples of animal origin the metabolite DDE normally represents more than 50 % of sum of DDT. A considerable lower contribution may indicate recent use of DDT. In contrast, samples of plant origin are generally dominated by the parent compound DDT.
- The concentrations determined in feed commodities including fish derived products generally are in the low µg/kg range and thus well below those that have been found to cause adverse effects in fish and domestic animals. However, it can not be excluded that elevated levels may be found in feed commodities that originate from areas where DDT has recently been or still is used.

Fate in animals and carry over

- Absorption at low doses (< 1 mg/kg b.w.) is almost complete in all investigated species provided that fat is present in the diet.
- The first step in the metabolism of DDT is the formation of DDD and DDE. These metabolites are usually converted to several hydroxylated compounds, and eliminated in a conjugated form in bile and urine. DDT, DDE and to a lesser extent DDD are lipophilic compounds which accumulate in adipose tissue.
- The half-life for DDT varies from 1 month in rats and dogs to 6 – 14 months in fish and four years in humans. DDE is generally more persistent in organisms than DDT.
- In ruminants transfer of DDT and related compounds to milk, expressed as a percentage of ingested dose, is about 5 %, 8 % and 26 % for *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE, respectively. The transfer of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE to the egg contents were 34, 3.5 and 42 %, respectively.
- Retention of DDT in fish can vary from 20 - 95 % of the dose depending on the concentration in the diet. The accumulation ratio calculated from the sum of *p,p'*-DDT and *p,p'*-DDE residues in adipose tissue relative to the *p,p'*-DDT levels in feed varied from 2.2 in sheep to 6 - 30 for broilers. The reported values for DDT only in beef cattle were in the 0.7 - 0.9 range whereas they were in the 11 - 26 range for DDE.

Human exposure

- Data from total diet studies, as well as from human milk monitoring programmes performed in various EU Member States, show a considerable decline of up to 90 % in human exposure to DDT and related compounds over the past three decades.
- Food of animal origin is the major source of human exposure to DDT and related compounds. Recent studies performed in some EU Member States indicate a mean dietary intake for adults and children of 5 - 30 ng/kg b.w. per day which is more than two orders of magnitude below the provisional tolerable daily intake (PTDI) of 0.01 mg/kg b.w..
- Recent exposure of breastfed infants was estimated to be around 0.001 mg/kg b.w..

DATA NEEDS AND RECOMMENDATIONS

- Besides the parent compound, *p,p'*-DDT, the analyses of feed samples should also include determination of *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE and *p,p'*-DDD because these compounds represent impurities in the technical mixtures, are major metabolites and are biological active.

- In the clean-up of samples, treatment with acids must be avoided in order to prevent the formation of DDE from dicofol, an authorised pesticide that is widely used and thus may be present in feed commodities.
- Proficiency tests performed on biological samples revealed large discrepancies in the performance of laboratories, indicating scope for improvement of the analytical methods.
- Toxicity data on some target animal species are lacking, however, given the relatively low levels identified in feed there does not seem to be an urgent need for additional toxicity studies.
- The Members States are requested by the Commission to report the results of their monitoring programmes on undesirable substances in animal feed as compliant or non-compliant only. The availability of detailed occurrence data concerning compounds and corresponding concentrations rather than condensed summary reports would be one prerequisite for an exposure assessment and identification of areas with an unusual high level of contamination. A European reporting system that facilitates these tasks should be set up.
- Given the large variation in the levels of DDT and related compounds in butter samples, it seems appropriate to collect more data from the regions where comparatively high levels have been reported.
- Special attention should be paid to the control of feed materials coming from areas of the world where DDT is still in use or has been used recently.

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DOCUMENTATION PROVIDED TO EFSA

Submission of occurrence data

Belgium. The Federal Agency for the Safety of the Food Chain.

Czech Republic. Central Institute for Testing and Supervising in Agriculture.

Denmark. Danish Plant Directorate.

Estonia. Agricultural Research Centre.

Finland. Plant Production Inspection Centre. Agricultural Chemistry Department.

Germany. Federal Office of Consumer Protection and Food Safety.

Iceland. Icelandic Fisheries Laboratories.

Norway. Norwegian Food Safety Authority, National Fish and Seafood Centre.

European Feed Manufacturers' Federation.

ANNEX. SURVEY OF LONG TERM TOXICITY STUDIES OF DDT AND RELATED COMPOUNDS IN DOMESTIC BIRDS

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Cockerels	4 weeks	Techn. DDT	125 (12.5 mg/kg b.w.)		24 weeks	Testis pathology	Balasubramaniam and Sundararaj, 1993
Cockerels	>5 weeks	Techn. DDT	100 (10 mg/kg b.w.)*		10-30 days	Inhibition of corticosteron syntesis	Srebocan and Pompe, 1970
Cockerels		<i>o,p'</i> -DDD	100 (10 mg/kg b.w.)*		10-30 days	Inhibition of corticosteron syntesis	
Cockerels		<i>p,p'</i> -DDT		100 (10 mg/kg b.w.)*	10-30 days	Inhibition of corticosteron syntesis	
Cockerels	>5 weeks	Techn. DDT	5 (0.5 mg/kg b.w.)		57 days	Reduced adrenal corticosterone and liver glycogen	Srebocan <i>et al.</i> , 1970
Chicken	8 days	<i>p,p'</i> -DDT	50 (5 mg/kg b.w.)		38 days	Immune depression	Radhakrishnan <i>et al.</i> , 1972
Male broilers	4 weeks	Techn. DDT		300 (30 mg/kg b.w.)	4 weeks	Signs of poisoning	Latimer and Siegel, 1974
Cocks	Mature	<i>p,p'</i> -DDT	100 (6 mg/kg b.w.)*		32 weeks	Reduced body weight, tremor	Arscott <i>et al.</i> , 1972
Cocks		<i>p,p'</i> -DDE	100-200 (6-12 mg/kg b.w.)*		32 weeks	Tremor	
Laying hens		DDT	310 (20 mg/kg b.w.)		12 weeks	Reduced egg production	Rubin <i>et al.</i> , 1947

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Laying hens		DDT		7.5 (0.5 mg/kg b.w.)	2 months	Red egg production and egg shell thickness	Smith <i>et al.</i> , 1970
Pullets		Techn. DDT	0.1 (0.006 mg/kg b.w)		10 weeks	Increased embryo mortality, red hatchability, egg production and thickness	Sauter and Steele, 1972
Pullets		<i>p,p'</i> -DDT		200 (12 mg/kg b.w)	12 weeks	Egg production and shell thickness	Davison and Sell, 1972
Pullets		<i>p,p'</i> -DDT		50 (3 mg/kg b.w.)	28 weeks	No effect on reproductive perform. but increased b.w.	Cecil <i>et al.</i> , 1972
		<i>o,p'</i> -DDT		50 (3 mg/kg b.w.)	28 weeks	No effect on reproductive perform. but increased b.w.	
		<i>p,p'</i> -DDE		50 (3 mg/kg b.w.)	28 weeks	No effect on reproductive perform. but increased b.w.	
Pullets		<i>p,p'</i> -DDT	5 + 50 (0.3 + 3 mg/kg b.w)		28 + 12 weeks	Reduced egg production	Cecil <i>et al.</i> 1972
		<i>o,p'</i> -DDT	5 + 50 (0.3 + 3 mg/kg b.w)			Reduced egg production	
		<i>p,p'</i> -DDE	5 + 50 (0.3 + 3 mg/kg b.w)			Reduced egg production	
Pullets		Techn. DDT		50 (3 mg/kg b.w.)	40 weeks	Reproductive performance	Cecil <i>et al.</i> , 1973
		DDT isomers		50 (3 mg/kg b.w.)	40 weeks	Reproductive performance	

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Pullets		<i>p,p'</i> -DDT		50 (3 mg/kg b.w.)	40 weeks	Reproductive performance	Cecil <i>et al.</i> , 1973
Hens	1 year	Techn. DDT	10 (0.6 mg/kg b.w.)		28 weeks	Reduced shell thickness	
		<i>p,p'</i> -DDT	10 (0.6 mg/kg b.w.)		28 weeks	Reduced shell thickness	
Hens		DDT		100 (6 mg/kg b.w.)	10 weeks	Reproductive performance	Scott <i>et al.</i> , 1975
Hens		Techn. DDT		300 (18 mg/kg b.w.)	84 days	Egg production and shell quality	Britton, 1975b
Hens		Techn. DDT	300 (18 mg/kg b.w.)		84 days	Induced microsomal enzymes	Britton, 1975a
Turkeys	6 weeks	<i>o,p'</i> -DDT		265 (16 mg/kg b.w.)*	15 weeks	Clinical effect	Simpson <i>et al.</i> , 1972
		<i>p,p'</i> -DDT		265 (16 mg/kg b.w.)*	15 weeks		
Japanese quail	25 days	Techn. DDT	100 (6 mg/kg b.w.)		55 days	Reduced survival when starved after exposure	Cross <i>et al.</i> , 1962
	50 days	Techn. DDT	100 (6 mg/kg b.w.)		30 days	Reduced survival when starved after exposure	
	50 days	Techn. DDT		100 (6 mg/kg b.w.)	30 days	Mortality during exposure	

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Japanese quail	Mature	<i>p,p'</i> -DDT		200 (12 mg/kg b.w)	60 days	Clinical and reproduction	Smith <i>et al.</i> , 1969
Japanese quail	Young	<i>p,p'</i> -DDT	100 (6mg/kg b.w.)*		45 days	Lag in egg prod., red shell strenght	Bitman <i>et al.</i> , 1969
		<i>o,p'</i> -DDT	100 (6 mg/kg b.w.)*		45 days	Lag in egg prod., red shell strenght	
Japanese quail	Young	<i>p,p'</i> -DDT	100 (6 mg/kg b.w.)*		74 days	Lag in egg prod., less shell Ca	Cecil <i>et al.</i> , 1971
		<i>p,p'</i> -DDE	100 (6 mg/kg b.w.)*		74 days	Lag in egg prod.	
Japanese quail	Young	DDT	100 (6 mg/kg b.w.)*		120 days	Reproductive and thyroid effects	Richert and Prahlad, 1972
		DDE	150 (9 mg/kg b.w.)*		120 days	Reproductive and thyroid effects	
		DDA		200 (12 mg/kg. b.w.)	120 days	Reproductive and thyroid effects	
Japanese quail		<i>p,p'</i> -DDT	15 (0.9 mg/kg b.w.)*		3 generations	Red egg production and fertility	Carnio and McQueen, 1973
Japanese quail	Mature	<i>p,p'</i> -DDT		100 (6 mg/kg b.w.)*	168 days	Reproductive performance	Robson <i>et al.</i> , 1976
		<i>p,p'</i> -DDE	100 (6 mg/kg b.w.)		168 days	Reduced body weight	
Japanese quail		DDT		5 (0.3 mg/kg.b.w.)	4 generations	Reduced hatchability	Shellenberger, 1978

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Japanese quail	Chicks	Techn. DDT		250 (15 mg/kg b.w.)	9 weeks	Adrenal effect	Biessmann and Von Faber, 1981
		<i>p,p'</i> -DDT	250 (15 mg/kg b.w.)*		5 weeks	Adrenal effect	
		<i>p,p'</i> -DDE	300 (18 mg/kg b.w.)*		5 weeks	Adrenal effect	
Japanese quail	Adult	<i>o,p'</i> -DDT	0.020 mg/bird per day*		120 days	Male reproductive function	El-Gawish and Maeda, 2005
Bobwhite quail	Mature	Techn. DDT		50 (3 mg/kg b.w.)	4 months	Liver weight, thyriod weight and function	Hurst <i>et al.</i> , 1974
Coturnix quail	7 days	DDE		50 (5 mg/kg b.w.)*	8 days	Behavioural effects	Kreitzer and Heinz, 1974
Mallard	Mature	<i>p,p'</i> -DDT		10 (0.6 mg/kg b.w.)	1 year	Reproductive effects	Heath <i>et al.</i> , 1969
		<i>p,p'</i> -DDE	10 (0.6 mg/kg b.w.)		1 year	Reproductive effects	
		Techn. DDD	10 (0.6 mg/kg b.w.)		1 year		
Mallard	Mature	<i>p,p'</i> -DDE		1 (0.06 mg/kg b.w.)	1/2 year	Mineral comp. of egg shell	Longcore <i>et al.</i> , 1971a
Mallard	Mature	DDT	75 (5 mg/kg b.w.)*		4 weeks	Shell gland	Kolaja and Hinton , 1976

Species/breed	Age	Compound	LOAEL mg/kg diet	NOAEL mg/kg diet	Duration	Crit. effect/effect studied	Reference
Mallard	Mature	DDT	50 (3 mg/kg b.w.)*		6 months	Shell gland	Kolaja and Hinton, 1978
American black ducks	Mature	<i>p,p'</i> -DDE	10 (0.6 mg/kg b.w.)		1/2 year	Red egg shell quality and reprod. tox	Longcore <i>et al.</i> , 1971b
Peking ducks	6 months	<i>p,p'</i> -DDE	250 (10 mg/kg b.w.)*		10 days	Red egg shell thickness	Peacall <i>et al.</i> , 1975
Pigeons	Mature	<i>p,p'</i> -DDT	150 (18 mg/kg b.w.)		42 days	Thyriod effects, liver weight	Jefferies and French, 1969
Ringdoves	Mature	<i>p,p'</i> -DDT	10 (0.6 mg/kg b.w.)*		4 weeks	Reduced blood estrogen and Ca in bones	Peacall, 1970
Ringdoves	Adult	DDE		2 (0.12 mg/kg b.w.)	8 weeks	Brain transmitters, liver weight	Heinz <i>et al.</i> , 1980

* = only one dose tested