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# Lindane

## MAK Value Documentation, supplement – Translation of the German version from 2019

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lindane; immunotoxicity; enzyme induction; liver; fertility; deveopmental toxicity; carcinogenicity; MAK value; maximum workplace concentration; skin absorption

## Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the maximum concentration at the workplace (MAK value) of lindane [58-89-9] and its classification in category 4 for carcinogenic substances considering all toxicological end points. In several studies an increased risk of non-Hodgkin lymphoma for lindane users in agriculture is described. After controlling for other pesticides, the risk decreased. Exposure or biomonitoring data are not available from these studies. From the viewpoint of the Commission, the studies are not sufficient to classify lindane as a human carcinogen. Because of its tumour-promoting effects on the liver of the rat and the liver carcinogenicity in the mouse, lindane is still classified in category 4 for carcinogenic substances. The critical toxic effects of lindane are immunotoxic and immunomodulating effects. After inhalation exposure, NOAECs of 0.6 mg/m$^3$ (rat) and 1 mg/m$^3$ (mouse) can be derived for histological changes of the spleen, thymus and bone marrow and NOAELs of 0.45 mg/kg body weight for rats and 2 mg/kg body weight for mice for immunological effects. Excluding skin contact, exposure to the MAK value of 0.1 mg/m$^3$ for the inhalable fraction results in a daily intake of 0.014 mg/kg body weight (100% absorption, 70 kg body weight and 10 m$^3$ respiratory volume) for humans. In this low concentration range, an inhibition of immunological responses is not likely and the MAK value is confirmed. Damage to the embryo or foetus is unlikely when the MAK value is not exceeded; therefore, the assignment to Pregnancy Risk Group C is confirmed as well. Skin contact may contribute significantly to systemic toxicity and the "H" notation is confirmed. Sensitization is not expected from the available data.

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|---|---|
| **MAK value (1998)** | **0.1 mg/m³ I (inhalable fraction) ≙ 0.0083 ml/m³** |
| **Peak limitation (2002)** | **Category II, excursion factor 8** |
| **Absorption through the skin (1966)** | **H** |
| **Sensitization** | **–** |
| **Carcinogenicity (1998)** | **Category 4** |
| **Prenatal toxicity (1998)** | **Pregnancy Risk Group C** |
| **Germ cell mutagenicity** | **–** |
| **BAT value (2000)** | **25 µg/l serum** |
| CAS number | 58-89-9 |
| **1 ml/m³ (ppm) ≙ 12.1 mg/m³** | **1 mg/m³ ≙ 0.083 ml/m³ (ppm)** |

Since the last documentation for lindane from 1998 (Greim 2001) and a supplement on peak limitation and its genotoxicity from 2002 (Greim 2002, available in German only) new data for several end points have been published that have made a re-evaluation of the classification and MAK value necessary.

The insecticidal effect of hexachlorocyclohexane (HCH) was discovered in 1935, and since 1942 γ-HCH (lindane) has been applied as an insecticide. In addition, it was used worldwide for the treatment of skin parasites, mainly scabies and lice, and as an additive in wood preservatives. Production and use peaked at the end of the 1960s; afterwards, production decreased. At the beginning of 2008, the use of lindane was banned in Europe under Regulation (EC) No. 850/2004. In the United States, the production of lindane was stopped in 1976 (ATSDR 2005), but large amounts of lindane were imported. In August 2006, the US EPA cancelled all registrations for the use of lindane in agriculture (US EPA 2006). However, it is still being used for the control of scabies and lice in the United States and probably also in India and Iran.

The synthesis of HCH from benzene and chlorine produces a mixture of isomers (technical HCH) consisting of 65% to 70% α-HCH, 7% to 20% β-HCH, 14% to 15% γ-HCH, 6% to 10% δ-HCH and 1% to 2% ε-HCH. Of these, only the γ-isomer is responsible for the insecticidal effect. The designation lindane is used for products consisting of at least 99% γ-HCH (UBA 2018). This documentation includes only those studies that were carried out with lindane (γ-HCH).

# Mechanism of Action

## Neurotoxicity

Acute intoxication with lindane causes central nervous symptoms. The alterations detected in the electroencephalogram (EEG) even include grand mal type seizures; these probably develop from disorders in the permeability of lipid-containing membranes. In addition, lindane has been shown to inhibit the absorption of chloride ions by the inhibitory synapses of the brain. This seems to be the primary mechanism of seizure induction by lindane. As lindane is structurally similar to picrotoxin, it binds to the picrotoxin binding site in the exterior part of the chloride ion channel. This blocks the effects of the neurotransmitter γ-aminobutyric acid (GABA), which mediates the entry of chloride into the channel. After a single intraperitoneal injection, dose-dependent GABA-inhibiting effects were observed in rats in vivo in a dose range from 5 to 40 mg/kg body weight. Furthermore, lindane increases the excitability of presynaptic cholinergic neurones in central and peripheral synapses. In nerve-muscle preparations, lindane concentrations of $5 \times 10^{-5}$ or $10^{-4}$ mol/l caused a massive increase in the release of the neurotransmitter acetylcholine.

In addition, lindane inhibits $Na^+/K^+$-ATPase, $Ca^{2+}/Mg^{2+}$-ATPase and $Mg^{2+}$-ATPase, which may affect calcium homoeostasis. Thus, the interplay of these effects leads to the excitatory activity in the central nervous system (Greim 2001).

### Change in cholinesterase activities

In albino mice, the butyrylcholinesterase activity was significantly reduced in the olfactory lobe and cerebrum following an acute intraperitoneal lindane dose of 40 mg/kg body weight (Bano and Bhatt 2010).

Groups of 16 male Wistar rats were given intraperitoneally 8 mg/kg body weight of lindane dissolved in DMSO, and a solvent control group was used concurrently. After 0.5 and 8 hours, 8 animals from each group were sacrificed. The acetylcholinesterase activity was significantly increased in the mitochondrial fraction of the cerebral cortex after 0.5 hours and in the thalamus and caudate nucleus after 4 hours (Vucević et al. 2009).

## Immunotoxic effects

Several reviews discussed various mechanisms of action for the immunotoxic effects of pesticides. An increase in oxidative stress, mitochondrial dysfunction, an increase in apoptosis, endoplasmic reticulum stress, esterase inhibition, an increase in autophagy and a disturbance in the ubiquitin-proteasome system were described (Mokarizadeh et al. 2015; Mrema et al. 2013). These mechanisms of action may be responsible for the increased incidence of non-Hodgkin's lymphomas in farmers and forestry workers. Some of these possible mechanisms of action have likewise been demonstrated for lindane.

### Oxidative stress/damage to mitochondria/apoptosis

Reactive oxygen species are formed during the metabolism of many pesticides. They may induce cytotoxicity through damage to proteins or the DNA, leading to disturbances in signal transduction and oxidative homoeostasis. Under experimental conditions, lindane forms toxic oxygen radicals. Evidence of oxidative stress is available from both in vivo and in vitro studies (Greim 2001).

Saccharomyces cerevisiae was exposed to 1.3 mM lindane for 2 hours. A more than 2-fold increase was found in the expression of various genes, such as ATX1, ARN1 and HSP26, which are related to oxidative stress and detoxification (Parveen et al. 2003).

Cytotoxicity, apoptosis and DNA fragmentation were investigated in the rat PC12 cell line (phaeochromocytoma cells) following incubation (6 to 72 hours) with lindane in concentrations of 0 to 5000 µg/l. No increase in cytotoxicity was observed up to the highest concentration tested. An increase in DNA fragmentation was found only at a lindane concentration of 10 µg/l after incubation in serum-free medium; this increase was not statistically significant. Likewise, at low concentrations, the slight, but not statistically significant, positive induction of various apoptotic factors (Bax, Bad, cytochrome C and caspase-3) was found. The authors concluded that lindane does not affect apoptosis in this cell line and DNA fragmentation was increased only in the low dose range (Aoki et al. 2008).

#### Results of animal studies

Male Wistar rats were given a single gavage dose of lindane of 5 mg/kg body weight (in olive oil). Apoptosis-relevant proteins such as cytochrome c, caspase-3 and caspase-9, Fas and FasL were investigated in the testes of the animals after 3, 6, 12, 24 and 72 hours. Increases in the levels of cytosolic cytochrome c, caspase-3 and caspase-9 were observed after 6 hours; the levels increased up to 24 hours after administration and then decreased, but did not return to the control values after 72 hours. The Fas and FasL levels in spermatogonia and spermatocytes also increased with time. At the same time, a decrease in the NF-κB p65 levels was observed in the cytosol after 3 hours with a maximum decline after 12 and 24 hours, whereas the levels in the nucleus increased. Likewise, the number of apoptotic cells increased with time (Saradha et al. 2009). This study revealed changes in proteins that are relevant to apoptosis.

Groups of 16 to 20 male Wistar rats were exposed for 8 weeks to lindane doses of 40 or 80 mg/kg diet (purity: 97%; about 3.6 or 7.2 mg/kg body weight; conversion factor: 0.09 according to EFSA (2012)) or to dichlorodiphenyltrichloroethane (DDT) doses of 100 or 200 mg/kg diet (purity: 95%; about 9 or 18 mg/kg body weight). The two substances were not investigated together. In addition, one group at each dose level was simultaneously given ascorbic acid. Thiobarbituric acid reactive substances (TBARS) in serum as markers of lipid peroxidation and the superoxide dismutase activity of the erythrocytes were investigated. The rats were immunized intraperitoneally with sheep erythrocytes 7 days before the end of exposure. There was a significant dose-dependent increase in TBARS and superoxide dismutase activity after the administration of both DDT and lindane. The antibody titres were significantly reduced in both dose groups after the administration of lindane and in the high dose group after the administration of DDT. The effects were attenuated by ascorbic acid. The authors assumed that the increased formation of reactive oxygen species may be the mechanism of action for immunotoxicity. In their opinion, this was supported by the negative correlation of the TBARS concentration and the increase in the activity of superoxide dismutase, with the antibody titres (Koner et al. 1998).

After an acute intraperitoneal lindane dose of 40 mg/kg body weight, significant decreases in the catalase activity and total protein and significant increases in the superoxide dismutase activity were observed in the cerebrum of male albino mice (Bano and Bhatt 2010).

Oxidative stress was investigated in the liver of male Sprague Dawley rats after a single intraperitoneal injection of lindane at a dose level of 50 mg/kg body weight. There was evidence of oxidative stress such as an increase in the DNA binding affinity of NF-κB in the liver, a decrease in glutathione and an increase in oxygen consumption and protein oxidation (Videla et al. 2004).

The study of Anilakumar et al. (2009) reported the induction of lipid peroxidation in the rat liver and an increase in the activity of anti-oxidative enzymes such as superoxide dismutase, catalase or glucose-6-phosphate dehydrogenase.

### Data in humans and human cell lines

The lymphocytes of healthy male non-smokers were exposed to lindane concentrations of 0.05 to 10 µg/l in vitro for 2 or 4 hours. The formation of reactive oxygen species, the mitochondrial membrane potential, caspase-3 activation and apoptosis were investigated. The increase in the formation of reactive oxygen species and the decrease in the mitochondrial membrane potential were statistically significant at the low concentration and above and were considerably more pronounced than after exposure to 1,2,4-trichlorobenzene and hexachlorobenzene. The increases in caspase-3 activity and the induction of apoptosis were statistically significant at concentrations of 5 µg/l and above (Michałowicz et al. 2013).

The percentage of cells positive for the anti-apoptotic protein Bcl-2 and PC-3 cells positive for the pro-apoptotic protein Bax were increased following incubation of the human breast-cell line MCF-7 with lindane in concentrations of $10^{-12}$ to $10^{-10}$ M for 24 hours without metabolic activation. MCF-7 cells are p53 wild-type cells, whereas PC-3 is a p53-deficient cell type. Incubation with high lindane concentrations ($10^{-4}$ M) led to an increase in the percentage of cells in the G1 phase and a decrease in the percentage of cells in the G2/M phase. The expression of the $p21^{WAF1/CIP1}$ protein, which is involved in cell cycle control, was increased in MCF-7 cells (Kalantzi et al. 2004).

Phosphorylation of histone H2AX was investigated as an epigenetic end point in various human cell lines (hepatoma cells HepG2, neuroblast cells Sh-Sy5Y, kidney fibroblasts ACHN and colon epithelial cells LS-174T). There was no difference between exposure to lindane and the solvent control (Graillot et al. 2012).

#### *Calcium homoeostasis*

Groups of 6 female Swiss mice were given lindane at dose levels of 0, 0.012, 0.12 or 1.2 mg/kg body weight with their diet. After 4 weeks, the calcium influx in lymphocytes increased by 17%, 31% and 14% compared with the levels determined in the control groups. The calcium influx decreased by 24%, 25% and 25% after exposure for 12 weeks and by 34%, 35% and 40% after 24 weeks. These changes in calcium influx correlated with the initial immunostimulation after 4 weeks and the subsequent immunosuppression after 12 and 24 weeks. Lymphocyte proliferation induced by lindane was in-

hibited by verapamil, a calcium channel blocker, and by trifluoperazine, a calmodulin inhibitor. The authors assumed that lindane modulates the calcium influx via calcium channels. Lindane inhibits the proliferation of lymphocytes by reducing phosphatidylinositol conversion, which results in disorganization of the membrane. In addition, lindane inhibits the $Ca^{2+}$-ATPase in rats, which leads to an increase in intracellular calcium levels. An increase in intracellular calcium levels may induce T cell activation (Meera et al. 1992, 1993).

### *Changes in gene expression, translocation t(14;18)*

The blood of 128 farmers who were exposed to various pesticides was investigated for translocation t(14;18) in activated B-lymphocytes and compared with that of 25 not exposed control persons from the same geographical region. The observation period was 9 years. The t(14;18)-positive clone increased by 253% in the exposed persons and by 87% in the control group. The increase in translocation in the control group was attributed to the age of the volunteers, whereas the increase in translocation in the group of exposed farmers could not be explained by aging effects. However, as only a few clones were carriers of the translocation in both groups, the authors concluded that the increase in translocation in the exposed farmers was caused by immunogenic effects of the pesticides used rather than by genotoxic effects. Environmental factors such as hepatitis C infection may have caused the increase in the control group. Furthermore, various factors that may be involved in the development of follicular lymphomas were investigated and compared with findings in the translocated clones of exposed persons or of the control group. The t(14;18) translocation, which is associated mainly with follicular lymphomas, is an important event in the malignant transformation of the lymphocytes. It permanently activates the Bcl2-coded gene, thereby preventing apoptosis. Another important factor in lymphomagenesis is the phase of somatic hypermutation. In this phase, single strand breaks occur in the DNA and enzyme activation-induced cytidine deaminase (AID) comes into play. The ongoing activity of this enzyme in lymphomas increases the genetic instability. These factors were detected in the clones of all three groups, but to varying degrees (Agopian et al. 2009).

### *Polymorphism of xenobiotic-metabolizing enzymes*

In a case–control study, the relationship between the incidence of non-Hodgkin's lymphomas and the polymorphism of 6 xenobiotic-metabolizing enzymes (CYP1A1, GSTT1, GSTM1, PON1, NAT1 and NAT2) was investigated in 169 patients with non-Hodgkin's lymphomas and compared with the findings from a control group without non-Hodgkin's lymphomas. In the non-Hodgkin's lymphoma group, the incidence of GSTT1-null and PON1 (paraoxonase A/A, A/B and BB) genotypes was significantly increased compared with the incidence in the control group (34% compared with 14% and 24% compared with 11%, respectively). In the non-Hodgkin's lymphoma patients, statistically significant increases in the adjusted odds ratios (ORs) of 4.27 (95% confidence interval (CI): 2.4–7.61) and 2.9 (95% CI: 1.49–5.72) were observed for the GSTT1-null and PON1-BB genotypes, respectively (Kerridge et al. 2002).

### *Induction of xenobiotic-metabolizing enzymes*

Groups of 10 male Wistar rats were given oral gavage doses of lindane of 0, 2.5, 5, 10 or 15 mg/kg body weight and day for 4 days. Two other exposure groups were given a lindane dose of 2.5 mg/kg body weight for 15 or 21 days. The total RNA and microsomes were investigated in the brain and lymphocytes 24 hours after the last administration. The activities of 7-ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dealkylase and *N*-nitrosodimethylamine demethylase and the protein levels of CYP1A1/1A2, CYP2B1/B2 and CYP2E1 were determined in the microsomes. To determine the activities of MAP-kinase and c-jun, another group of rats was given a lindane dose of 15 mg/kg body weight for 120 minutes and interim sacrifices were carried out every 15 minutes. Lindane induced both dose-dependent and time-dependent increases in the activities of ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dealkylase and *N*-nitrosodimethylamine demethylase and increases in the protein and RNA levels of CYP1A1, 1A2, 2B1/B2 and 2E1 in the brain and lymphocytes. The increases were statistically significant at a dose level of 10 mg/kg body weight and above. Induction in the brain was about twice as high as in the lymphocytes. The ERK1 MAP-kinase and c-jun activities increased only

during the first 75 and 90 minutes, respectively, whereas the ERK2 MAP-kinase activity was not increased either in the brain or in the lymphocytes. There was no change in the phosphorylation of p38 (Khan et al. 2013).

Human cell lines (JEG-3 cells and transfected human embryonic kidney 293 cells) were incubated with lindane in concentrations of 0, 25, 50 or 75 µM for exposure periods ranging from 10 minutes to 18 hours. Cytotoxicity was not observed. After incubation for 10 or 30 minutes, the aromatase activity increased in a concentration-dependent manner, and incubation for 30 minutes or 1 to 18 hours induced inhibitory effects that were dependent both on concentration and time. This study demonstrated in vitro the modulation of aromatase activity, which might lead to a disturbance in oestrogen biosynthesis (Nativelle-Serpentini et al. 2003).

### *Genetic changes associated with lindane*

Lindane was associated with diffuse large B-cell lymphomas in agricultural workers (field crop and vegetable farm workers, and general farm workers) and with chronic lymphocytic leukaemia or small lymphocytic lymphomas in farm workers ('t Mannetje et al. 2016) (see also Section "Carcinogenicity" under "Effects in Humans"). Both types of lymphoma exhibit specific genetic changes (Blombery et al. 2015), and several of them were detected in Jurkat T cells exposed to lindane (6-hour exposure to 130 µM lindane). In Jurkat cells, for example, exposure induced an over-expression of Bcl6, which like Bcl2 is translocated during lymphomagenesis. Over-expressed PELI1 is involved in the activation of the NF-κB signalling pathway. Over-expressed protein phosphatase DUS10 regulates the MAP-kinase activity by dephosphorylating p38. Inhibition of p38 phosphorylation was found to reduce Bcl-2 expression (Shao et al. 2013). These studies suggested that exposure to lindane leads to genetic changes; however, it is not possible at present to assess their relevance in the pathogenesis of lymphoma development.

## Toxicokinetics and Metabolism

The 1998 documentation (Greim 2001) reported 50% absorption by inhalation. However, the original reference does not offer any evidence to substantiate this figure, and a comprehensive review of the data did not establish the quantitative absorption of lindane (ATSDR 2005). In rats, the amount of lindane orally absorbed from technical HCH was found to be 99.4% (ATSDR 2005). Dermal absorption depends on the solvent (Greim 2001).

The studies published in the meantime with epicutaneous application in humans are described below. A commercially available wood preservative containing 3 mg lindane per ml white spirit was applied to 75 $cm^2$ of the forearm of 4 volunteers in an amount of 1 ml, the application site was washed after 6 hours and the stratum corneum was tape-stripped. After 6 hours, the absorption of 60% was determined from the difference between the amount applied and the sum of amount washed off and included in the stratum corneum. The peak concentration of lindane in the plasma was 0.47 ± 0.14 µg/l. The elimination half-life was 25 to 58 hours. Less than 1% of the applied dose was detected in the 72-hour urine in the form of metabolites; these were 2,4,6-, 2,3,5- and 2,4,5-trichlorophenol glucuronides (Dick et al. 1997). An absorption rate of 4 µg/$cm^2$ and hour was calculated from these values. Therefore, 8 mg would be absorbed under standard conditions (1 hour; 2000 $cm^2$ of skin). A PBPK model was established using the data of Dick et al. (1997), and an initial permeability constant of $1.52 \times 10^{-3}$ cm/hour and a final permeability constant of $1.33 \times 10^{-4}$ cm/hour were derived (Sawyer et al. 2016). A similar study using acetone as the solvent yielded the dermal absorption of 10% following the application of 120 mg of lindane. However, application in acetone is not a route of exposure typical for the workplace (Dick et al. 1997).

# Effects in Humans

## Case studies

A 37-year-old woman used a lotion containing lindane for the treatment of scabies at intervals of 10 and 3 days. Following the third application to her upper body, face and under her arms, she did not wash the lotion off the following day. After 18 hours, she experienced initial symptoms of the central nervous system, such as motor tics, uncontrolled shaking and difficulties in speaking and thinking. She developed paraesthesia in her hands and feet as well as myoclonic convulsions. In addition, severe nausea occurred. Incontinence, acute rhabdomyolysis and central nervous hyperactivity with auditory and visual hallucinations developed 8 days after treatment with muscle relaxants. More than one year after treatment with high doses of Valium, the patient was considered to have recovered and medication with Valium was no longer required. This case is one of the most severe intoxications described for lindane (Hall and Hall 1999).

In the period from 1997 to 2001, 20 patients admitted to hospital with lindane intoxication were treated with an acetylcholine inhibitor (atropine) and received medical care for 7 to 21 days. A group of 20 healthy persons who were not exposed to lindane or other chlorinated organic pesticides was used as the control. Blood samples were taken within 24 hours and 10 days and examined for IgG, IgM, IgA, IgE and cytokines (IL-2, IL-4, TnF-α and IFN-γ). The lindane concentrations in the blood were 0.25 to 1.3 mg/l. Lindane was not detected in the blood of the control persons. There were no unusual findings in the haematological and biochemical parameters that were routinely investigated in the patients. The decrease in the acetylcholinesterase activity observed in the patients was not statistically significant compared with that in the control persons. The serum immunoglobulin concentrations were about the same in the patients and in the control persons. In the patients, the IL-2, IL-4 and TNF-α cytokine levels were significantly increased and positively correlated with the lindane concentration in the blood. The IFN-γ levels were significantly reduced. Atropine is not known to have effects on the cell-mediated or humoral immune response (Seth et al. 2005).

## Allergenic effects

There are no new data available.

## Reproductive and developmental toxicity

### Fertility

The sperm of 50 fertile and 50 infertile men were investigated as regards their number, viability and motility and for Y-chromosomal microdeletions (Yq microdeletions). In addition, the concentrations of HCH isomers were determined in the semen. No significant differences in the concentration of lindane (γ-HCH) were observed in the semen of fertile and infertile men. In the infertile men, the sum of all HCH isomers was increased and the sum of β-HCH was significantly increased. The total sperm count, the number of sperm per ml, their viability and motility were significantly reduced in the group of infertile men compared with the sperm parameters in the fertile men. Yq microdeletions were not found in the fertile men, while they occurred in 20% (10/50) of the infertile men. The group of infertile men was subdivided further for oligospermia, asthenospermia, oligo-asthenospermia and azoospermia. In the group with asthenospermia, the lindane concentration correlated significantly with the reduced sperm count (Khan et al. 2010). As the lindane concentrations in the semen of fertile and infertile men did not differ significantly, the effects were not caused by lindane. However, there may have been effects caused by the total concentration of all isomers.

The sperm of 60 fertile and 150 "infertile" men (their female partners failed to become pregnant within one year) were examined depending on the lindane concentrations in the semen. The volunteers were divided into 4 concentration groups: control persons (53 men; < 5.32 µg lindane/l), low exposure (53 men; 5.32–15.67 µg/l), medium exposure (52 men; 15.68–18.9 µg/l) and high exposure (52 men; 19–39.04 µg/l). Sperm motility was reduced at concentrations of 5.32 µg/l and

above, but this effect was not dependent on the concentration. Changes in the colour, odour, viscosity, pH or volume of the semen were not observed. The sperm of 12 healthy men were exposed to different lindane concentrations for periods ranging from 30 minutes to 96 hours (no other details). After 12 hours, sperm motility decreased significantly in a concentration-related manner (Pant et al. 2013).

An epidemiological study included 85 fertile men and 193 "infertile" men whose female partners failed to become pregnant within one year. The groups were made up of persons of the same age and with the same eating and smoking habits. The lindane concentrations in the semen of the "infertile" men were significantly increased compared with those of the fertile men (fertile: 3.1 ± 1.37 µg/l; "infertile": 18.64 ± 6.36 µg/l), as were the reactive oxygen species, lipid peroxidation and sperm with depolarized mitochondria. Sperm motility and the sperm concentration were significantly reduced in the "infertile" men (Pant et al. 2014). As the results for p,p′-dichlorodiphenyl dichloroethene (DDE) were the same, it remains unclear whether the effects were caused by lindane alone. However, it is probable that chlorinated organic compounds have an effect on male fertility.

### In vitro

Sperm were incubated with lindane concentrations of 0, 0.1, 1, 5, 15 and 30 µM for 15 minutes. Progesterone or the solvent control was then added and incubation was allowed to proceed for 60 minutes. At the low concentration and above, lindane led to the dose-dependent inhibition of the spontaneous and progesterone-stimulated acrosome reaction and increased the calcium influx into the sperm (Silvestroni and Palleschi 1999).

Sperm of healthy men were incubated with lindane concentrations of 0, 0.03, 0.3 or 3 µg/l for 5 hours. Sperm motility, sperm viability and the calcium-dependent sperm acrosome reaction remained unaffected (Pflieger-Bruss et al. 2006).

### Endometriosis

In a cohort study with 473 working women aged between 18 and 44 years, 190 of whom had endometriosis, a statistically significant association was observed between the occurrence of endometriosis and the lindane concentration in the adipose tissue (Buck Louis et al. 2012). It is unclear whether there is a causal relationship between lindane and the occurrence of endometriosis.

### Developmental toxicity

Environmental studies that investigated a relationship between chlorinated organic substances in the blood, cord blood or placenta and effects on the duration of pregnancy, birth weight and the crown-heel length at birth, and delayed growth or malformations in newborn infants were not included in the evaluation of developmental toxicity because the persons were exposed to more than one substance (Fenster et al. 2006; Fernandez et al. 2007; Gerhard et al. 1998; Siddiqui et al. 2003; Tyagi et al. 2015).

## Genotoxicity

The blood of 50 women (non-smokers) working in agriculture in Mexico and the cord blood of their newborn infants were investigated for pesticides and the formation of micronuclei and DNA damage (comet assay; monocytes). The women themselves did not use the pesticides. The lindane concentration in the cord blood of 995 ng/g blood lipids (median; interquartile range: 695–1193 ng/g blood lipids) was more than twice as high as that in the blood of the mothers (median: 391 ng/g blood lipids; interquartile range: 252–638 ng/g blood lipids). Likewise, all other pesticide concentrations, with the exception of the DDE level, were much higher in the cord blood than in the maternal blood. The frequency of micronuclei was significantly higher in the maternal blood than in the cord blood. No effects were observed on the formation of nucleoplasmic bridges or chromatin buds. There was a statistically significant increase in the DNA strand break frequency in the cord blood cells compared with that in the maternal blood cells. The increase did not correlate with the lindane concentration (Alvarado-Hernandez et al. 2013).

In a longitudinal study in 210 workers (28% smokers; 40% alcoholics) who had worked in agriculture in India for 1 to 25 years, blood samples were investigated for DNA damage (comet assay) between December 2003 and January 2006. Every agricultural worker was his own control. The control value was established 5 to 6 months after the first blood sample was taken; the exposure concentration was low during this period (no other details). In addition, 50 healthy men adjusted for age (non-smokers) were evaluated as a control group. DNA damage was found in 35.7% of the exposed agricultural workers, but only in 8% of the age-adjusted control group. The cases with an increase in DNA damage decreased to 25% in the period with low exposure (Kaur et al. 2011). This study has not been included in the evaluation of the genotoxicity of lindane because the exposure was not differentiated according to different pesticides and no data are available for exposure levels.

## Carcinogenicity

### Case–control studies

In a population-based case–control study in the United States with interviews of farmers, the data set included 987 cases of non-Hodgkin's lymphomas and 2895 control persons. Adjustment was made for age, residence, and direct or proxy interviews. A statistically significant increase in the OR of 1.5 (95% CI: 1.1–2) was found for non-Hodgkin's lymphomas (93 cases/151 controls) in farmers who reported having been exposed to lindane at some time, but no adjustment was made for other pesticides. After adjustment for various pesticides, the risk was still increased with the exception of a few cases. The risk was 1.4 (95% CI: 0.5–4.3) for farmers who had first used lindane more than 20 years before the study and were exposed for 4 days or less. Those who were exposed for 5 days or longer had a risk of 2.5 (95% CI: 0.6–11.7). However, the incidences of 11 and 5 exposed cases are so small that reliable conclusions cannot be drawn. The risk increased to 3.3 (95% CI: 0.8–13.8) in farmers who were exposed for more than 10 days. There is no explanation of why the risk in farmers who were interviewed directly (78 cases/123 control persons) was lower (1.3; 95% CI: 0.9–1.8) than in those with proxy respondents (14 cases/27 control persons; 2.1; 95% CI: 1.0–4.4). The authors concluded that the development of non-Hodgkin's lymphomas is not strongly associated with exposure to lindane, but that the involvement of lindane cannot be ruled out. Pesticide users have an increased risk of developing non-Hodgkin's lymphomas; in this study, the increase in the risk could not be attributed to a specific pesticide (Blair et al. 1998).

As the incidence of non-Hodgkin's lymphomas in Canada had increased during the preceding 25 years, a population-based case–control study was carried out among pesticide users who were exposed to pesticides, mixtures or individual substances for more than 10 hours a year. Complete interviews were obtained from 179 persons with non-Hodgkin's lymphomas and 456 control persons; 15 and 23 of these users, respectively, were exposed to lindane. However, the evaluation itself was based on 517 cases and 1506 control persons; the reasons for this were not explained in the publication. The increase in the OR was 2.06 (95% CI: 1.01–4.22) even after adjustment for measles, mumps, cancer, a history of cancer in the family and age and was thus statistically significant. Both a misclassification of the pesticide exposure and a recall bias are possible. As the authors did not report why the evaluation was not based on only those cases and control persons for whom complete interviews were available, the validity of the study is restricted.

In a case–control study in a total of 266 sheep farmers in Iceland, the relationship between exposure to HCH isomers and the risk of developing non-Hodgkin's lymphomas was investigated for the period between 1962 and 2003 (45 cases of non-Hodgkin's lymphomas and 221 control persons). A mixture of HCH isomers (Gammatox®; technical HCH) had been used to control ectoparasites (mites) in sheep since 1947. As of the middle of the 1970s, the solution consisted only of lindane. Adjustment was made for the age of the sheep farmers. As no other insecticides or herbicides were used for sheep dipping, it is assumed that the sheep farmers were exposed only to HCH isomers. The number of dipped sheep was used as a surrogate for exposure to lindane. Sheep farmers who dipped fewer than 100 sheep were used as the control group. Depending on the number of sheep and after adjusting for the age of the sheep farmers, the ORs were 3.83 (95% CI: 1.58–9.31) for farmers who treated 100 to 199 sheep and 3.44 (95% CI: 1.31–9.04) for farmers who dipped 200 sheep or more. The authors discussed the small number of cases and other possible confounders for which adjustment was not made, such as genetic factors, immunodeficiency, lifestyle factors and other occupational exposures. How-

ever, they concluded that the threefold increase in the risk of developing non-Hodgkin's lymphomas in these sheep farmers is linked with HCH isomers (Rafnsson 2006). As a pure lindane solution was used only as of the mid-1970s, exposure to a mixture of various isomers has to be assumed for the time before. In addition, a control group without exposure was not investigated. Therefore, the study provides only weak evidence of an increased risk of developing non-Hodgkin's lymphomas.

Epilymph case–control study: Between 1998 and 2003, 2348 lymphoma cases were reported among a total of 4810 pesticide users from 6 European countries (Spain, France, Germany, Italy, Ireland and the Czech Republic). Data for the exposure levels, period of exposure, and type and use of different pesticides were obtained from interviews. In addition, data were collected for sociodemographic factors, lifestyle, state of health and workplaces at which the persons were employed for 1 year or longer. On the basis of these data, 4 exposure groups were defined: no exposure, low exposure (< 50 days/year), medium exposure (51–100 days/year) and high exposure (> 101 days/year). Cumulative exposure was determined for each group of pesticides. In the group of chlorinated organic compounds that included lindane, no increase in the lymphoma risk (cases: 33; control persons: 37; OR: 0.9; 95% CI: 0.6–1.5) or B-cell lymphomas (cases: 27; control persons: 37; OR: 0.9; 95% CI: 0.5–1.4) was observed; there was no statistically significant increase in the risk of developing chronic lymphocytic leukaemia (cases: 10; control persons: 37; OR: 1.2%; 95% CI: 0.6–2.5). The risks did not increase with cumulative exposure (Cocco et al. 2013). Therefore, this study does not provide evidence of an increase in the lymphoma risk after exposure to chlorinated organic pesticides.

Meta-analysis: In the following, one of the largest meta-analyses of 10 international studies is described, which evaluated the relationship between the incidence of 4 different subtypes of non-Hodgkin's lymphomas and various occupations. No information was provided on the specific chemicals to which the workers were exposed. The InterLymph Consortium analysed a total of 10 046 cases and 12 025 control persons. The diagnoses of diffuse large B-cell lymphoma (DLBCL; 3061 cases), follicular lymphoma (FL; 2140 cases), chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL; 1014 cases) and peripheral T-cell lymphoma (PTCL; 632 cases) were made between 1988 and 2004. Three exposure groups were formed according to exposure periods of < 1 year, 1 to 10 years and > 10 years. Adjustment was made for age, sex, study centre and smoking habits, but smoking was not regarded as a confounder. The results were reported for workers who had been employed for more than 10 years in their specific occupation. The ORs for all non-Hodgkin's lymphomas were 1.26 (95% CI: 1.05–1.51) for persons who had been employed as field crop and vegetable farm workers at some time and 1.19 (95% CI: 1.03–1.37) for general farm workers. Positive associations were observed for the occurrence of DLBCL in crop farmers and forestry workers. Likewise, positive associations were found in crop farmers for CLL/SLL and PTCL, but not for follicular lymphomas. However, positive associations were not found for animal farming ('t Mannetje et al. 2016).

## Cohort studies

Agricultural Health Study: A cohort study of 54 306 pesticide users in North Carolina and Iowa evaluated the risk of developing non-Hodgkin's lymphomas between 1993 and 1997. Information about exposure was obtained from questionnaires. The first interview included questions about exposure to 50 pesticides at some time or never and about the duration and frequency of application. A total of 25 291 users participated in the second interview (exposure to 25 pesticides). The follow-up period lasted until 2005.

Regarding the evaluation of lindane, two publications are available: Purdue et al. (2007) and Alavanja et al. (2014) which are described in the following.

A total of 523 cases of non-Hodgkin's lymphomas were diagnosed, 85 of these in the group of users exposed to lindane at some time and 396 cases in the group of persons who were not exposed to lindane. The relative risk was 1 (95% CI: 0.8–1.2) and was therefore not increased. When the evaluation was carried out according to the frequency of application in days (lifetime exposure), the increase in the relative risk of 2.5 (95% CI: 1.4–4.4) was statistically significant in the group of users exposed for more than 56 days (up to 457 days). A relative risk of 1.8 (95% CI: 1.0–3.2) was determined when the frequency of application in days (lifetime exposure) and the intensity of application were taken into account. As there were only 14 cases in both groups, the number of cases is regarded as very small. If adjustment was made for

DDT, the risk was still increased, but the increase was no longer statistically significant. The risk levels calculated for different types of lymphoma are shown in Table 1.

**Tab. 1** Relative risk levels for different types of lymphoma (Alavanja et al. 2014)

| Types of lymphoma | Ever/never exposed | Relative risk (95% CI) |
|---|---|---|
| sum of: small cell lymphocytic lymphoma/ chronic lymphocytic leukaemia/mantle cell lymphoma | 27/113 | 1.2 (0.60–1.5) |
| large B-cell lymphoma | 12/95 | 0.6 (0.30–1.1) |
| follicular B-cell lymphoma | 16/41 | 1.7 (0.96–3.2) |
| multiple myeloma | 13/73 | 1.1 (0.50–2.0) |

The authors assumed that there is an association between exposure to lindane and the development of non-Hodgkin's lymphomas; this needs to be clarified in more detail (Alavanja et al. 2014).

After evaluation of the data, Purdue et al. (2007) reported increased risks of developing non-Hodgkin's lymphomas after exposure to lindane of 1.9 (95% CI: 0.8–4.7) in the group with lifetime exposure of 1 to 22 days and of 2.1 (95% CI: 0.8–5.5) in the group exposed for 22 days or longer. Adjustment was made for age, US state, sex, education, smoking, alcohol consumption, family history of cancer and days of exposure to other pesticides. If the intensity of lindane use was included in the evaluation (information from the participants or data from the literature), relative risks of 1.6 (95% CI: 0.6–4.1) or 2.6 (95% CI: 1.1–6.4) were calculated.

Meta-analysis: A meta-analysis of 37 epidemiological reports evaluated the relationship between exposure to chlorinated organic pesticides and an increased risk of developing non-Hodgkin's lymphomas. Criteria for the selection of the studies were exposure to DDT, DDE, hexachlorobenzene, HCH and chlordane, evidence of exposure determined in body fluids or organs, non-Hodgkin's lymphomas as a study end point, reported ORs or relative risks (RR) with confidence intervals or detailed information to extrapolate these values. A statistically significant increase in the risk of 1.4 (95% CI: 1.27–1.56) was calculated for non-Hodgkin's lymphomas on the basis of 13 studies. The meta-analysis yielded statistically significant increases in the risk of developing non-Hodgkin's lymphomas for the individual substances DDE (OR: 1.38; 95% CI: 1.14–1.66), HCH (OR: 1.42; 95% CI: 1.08–1.87), hexachlorobenzene (OR: 1.54; 95% CI: 1.20–1.99) and chlordane (OR: 1.93; 95% CI: 1.51–2.48). After exposure to DDT, the increase in the risk of developing non-Hodgkin's lymphomas was not statistically significant (OR: 1.02; 95% CI: 0.81–1.28) (Luo et al. 2016).

Another meta-analysis was carried out using the results from the studies of Blair et al. (1998), McDuffie et al. (2001), Purdue et al. (2007) and Rafnsson (2006). A statistically significant increase in the risk of developing non-Hodgkin's lymphomas of 1.6 (95% CI: 1.2–2.2) was derived for users exposed to lindane (Schinasi and Leon 2014).

### Relationship between contact with farm animals (poultry, swine, cattle, sheep) and developing non-Hodgkin's lymphomas

It is assumed that 30% of all cancers occurring in developing and tropical countries are caused by infections compared with 10% in industrial countries (Efird et al. 2014).

Another analysis of the findings of the Agricultural Health Study (see above) examined the relationship between the cancer risk for farmers and the number of animals raised on their farm, in particular poultry. The farmers were divided according to the type and number of animals on their farms and activities related to raising animals, performing veterinary services, butchering, grinding feed, milking cows and working in poultry or swine confinement areas. In addition, risk factors such as smoking, diet, history of cancer, medical conditions and demographic information were reported and taken into account. Non-smokers accounted for 57% of the farmers producing and raising animals. The risk of developing non-Hodgkin's lymphomas was increased for farmers raising poultry (23 cases; RR: 1.6; 95% CI: 1.0–2.4) and for farmers raising swine (RR: 1.6; 95% CI: 0.6–4.3). A statistically significant increase in the risk of developing non-Hodgkin's lymphomas of 2.1 (95% CI: 1.2–3.7) was established for farmers working in a poultry confinement

area and of 3.6 (95% CI: 1.2–10.3) for farmers working in a swine confinement area. The risk did not increase with the number of animals. No association was established for other cancers (Beane Freeman et al. 2012). The authors referred to other studies (Cano and Pollán 2001; Fritschi et al. 2002; Svec et al. 2005; Tranah et al. 2008) that described an association between the occupational contact with animals and an increase in the risk of developing non-Hodgkin's lymphomas (Beane Freeman et al. 2012).

### Summary

Several studies determined an increased risk of developing non-Hodgkin's lymphomas for users of lindane. The risk increased with the length and intensity of application. Exposure levels were not recorded and biomonitoring was not carried out in any of the studies. The risks decreased if adjustment was made for other pesticides, mainly chlorinated organic pesticides. The authors discussed whether lindane may have contributed to the development of non-Hodgkin's lymphomas; this requires further research. As animal studies provided evidence that lindane causes immunomodulating effects, an association would be plausible on the basis of the mechanism of action. However, it has to be taken into account that when the findings of the Agricultural Health Study were evaluated according to the type of livestock, also farmers raising poultry and working in a poultry confinement area had a significantly increased risk of developing non-Hodgkin's lymphomas. Therefore, livestock is a considerable confounder. In the absence of information on the level of exposure and on possible confounders, such as other chlorinated organic pesticides and livestock, the data are not sufficient to confirm that lindane induces carcinogenic effects in humans.

## Animal Experiments and in vitro Studies

### Subacute, subchronic and chronic toxicity

In order to re-evaluate the previous MAK value of 0.1 mg/m$^3$, the results of the 3-month inhalation studies in rats and mice and the feeding studies that investigated immunotoxic effects are discussed in detail again (see also Greim 2001).

#### Inhalation

In a 3-month inhalation study with a 6-week recovery period, groups of 12 male and 12 female Wistar rats were exposed whole-body to lindane in concentrations of 0, 0.02, 0.12, 0.6 or 4.5 mg/m$^3$ for 6 hours a day. Only in the group exposed to 4.5 mg/m$^3$ were there transient mild clinical effects (poorer general condition, diarrhoea and piloerection) and markedly increased hepatic cytochrome P450 values, which returned to normal during the 4-week recovery period. The numerous functional, laboratory diagnostic and morphological tests revealed no effects. The concentrations of lindane in the serum, liver, brain and adipose tissue increased linearly as the concentration increased; the highest levels were found in serum and were 390 µg/kg serum in males and 270 µg/kg serum in females. The NOEL (no observed effect level) found in this study was 0.6 mg/m$^3$ (CIEL 1983).

Groups of 45 male and 45 female CD-1 mice were exposed whole-body to lindane in concentrations of 0, 0.25, 1 and 5 or 10 mg/m$^3$, for 6 hours a day, on 5 days a week, for 13 weeks. After 2 male and 12 female mice from the group exposed to 10 mg/m$^3$ died in the first week of the study, the concentration was reduced to 5 mg/m$^3$ beginning on week 2; another 3 females and 3 males in this group died. Of the mice exposed to 1 mg/m$^3$, 1 female and 1 male mouse died between weeks 3 and 20; the causes of death were not established. Changes in clinico-chemical or haematological parameters were not detected. Nor were there any histopathological changes. Likewise, additional myelography revealed no changes up to an exposure concentration of 1 mg/m$^3$ (see Table 2). The serum lindane concentrations were 41, 85 and 290 µg/l in the female animals in week 14; the concentrations in the females were about twice those in the males. As the causes of death in the group exposed to 1 mg/m$^3$ could not be established, the NOAEC (no observed adverse effect concentration) was reported to be 0.3 mg/m$^3$ (CIEL 1988). Cytochrome P450 levels were not determined.

**Tab. 2** Evaluation of the myelogram after exposure of CD-1 mice to lindane by inhalation (CIEL 1983, 1988)

| Concentration, duration | Findings |
|---|---|
| 0, 0.3, 1, 10 (only in week 1), 5 mg/m³, 14 weeks, lindane (technical grade: 99.6% purity) | **0.3 mg/m³:** NOAEC<br>**1 mg/m³ and above:** mortality increased, ♀: serum urea nitrogen level ↑ (9%, 33%, 44%)<br>**5 mg/m³:**<br>week 7: ♂: sternum megarubricytes ↑, total erythrocytes ↑, total eosinophils ↑, progranulocytes ↓, ♀: eosinophilic myelocytes and metamyelocytes: ↓<br>week 14: ♂ and ♀: lymphocyte count ↓, lymphocyte count ↑ (femur), rubiblasts, polychromatophilic cells ↑<br>⟶ no time-dependency, inconsistencies in the statistical significance within one sex and between the sexes ⟶ not induced by the test substance<br>week 20: ♀: potassium in the urine ↑ |

## Oral administration

### Immunotoxic effects

#### *Rat*

Male and female **Charles Foster rats** were given oral lindane doses of 0, 6.25 or 25 mg/kg body weight daily for 35 days and intramuscular injections of Salmonella typhimurium and Salmonella paratyphimurium A and B antigens on days 7 and 14; the specific antibody titre was reduced by the treatment with lindane (Dewan et al. 1980).

Young **Wistar rats** were given lindane in concentrations of 0, 5, 20 or 30 mg/kg diet (about 0.45, 1.8 or 2.7 mg/kg body weight and day; conversion factor: 0.09 according to EFSA (2012)) for up to 22 weeks following a subcutaneous injection of 0.2 ml tetanus toxoid in Freund's complete adjuvant 20 days before testing. The body weights, spleen and thymus weights, feed consumption and survival were not affected. At a lindane concentration of 5 mg/kg diet, the concentrations of toxoid-specific antibodies were slightly, but not significantly reduced. At 20 mg/kg diet and above, the titres of toxoid-specific antibodies and those of total IgM and IgG were significantly reduced and the migration of leukocytes and peritoneal macrophages inhibited (see Table 3) (Saha and Banerjee 1993). The authors concluded that the immunomodulating effects correlated more with time than with the administered dose.

**Tab. 3** Immunotoxic effects in Wistar rats after oral administration of lindane with the diet (Saha and Banerjee 1993)

| Dose [mg/kg body weight and day] | 8 weeks | 12 weeks | 18 weeks | 22 weeks |
|---|---|---|---|---|
| 0.45, NOAEL | no effects on antibodies, thymus or spleen weights | specific antibodies ↓ (n. s.) | specific antibodies ↓ (n. s.) | specific antibodies ↓ (n. s.) |
| 1.8 | specific antibodies ↓ (n. s.) | specific antibodies ↓* | specific antibodies ↓*, no increase with time | specific antibodies ↓*, slight increase with time |
| | | migration of leukocytes, peritoneal macrophages ↓* | migration of leukocytes, peritoneal macrophages ↓*, time-dependent increase | migration of leukocytes, peritoneal macrophages ↓*, time-dependent only in leukocytes |
| | | | | increase in total IgM and IgG ↓* |
| 2.7 | specific antibodies ↓ (n. s.) | specific antibodies ↓* | specific antibodies ↓*, no increase with time | |
| | migration of peritoneal macrophages ↓* | migration of peritoneal macrophages ↓*, time-dependent increase | migration of peritoneal macrophages ↓*, no time-dependent increase | |
| | migration of leukocytes ↓* | migration of leukocytes ↓*, no time-dependent increase | | migration of leukocytes ↓*, time-dependent increase |
| | | | increase in total IgM and IgG ↓* | |

NOAEL (no observed adverse effect level); n. s.: not significant
*$p < 0.05$

Groups of 16 to 20 male **Wistar rats** were exposed for 8 weeks to lindane doses of 40 or 80 mg/kg diet (purity: 97%; about 3.6 or 7.2 mg/kg body weight and day; conversion factor: 0.09 according to EFSA (2012)) or to DDT doses of 100 or 200 mg/kg diet (purity: 95%; about 9 or 18 mg/kg body weight and day; conversion factor: 0.09 according to EFSA (2012)). The two substances were not investigated together. In addition, one group at each dose level was simultaneously given ascorbic acid. The rats were immunized intraperitoneally with sheep erythrocytes 7 days before the end of exposure. The antibody titres were significantly reduced in both dose groups after administration of lindane and in the high dose group after administration of DDT. All effects were attenuated by ascorbic acid. The authors assumed that the increased formation of reactive oxygen species was the possible mechanism of immunotoxic effects. In their opinion, this was supported by the negative correlation between the increased TBARS formation and the increased superoxide dismutase activity, on the one hand, and the antibody titres, on the other hand (Koner et al. 1998).

### *Mouse*

Groups of 6 female **Swiss mice** were given lindane with the diet in doses of 0, 0.012, 0.12 and 1.2 mg/kg body weight and day for a maximum of 24 weeks. Both the cell-mediated immune response (delayed type hypersensitivity reaction: induction and provocation with sheep erythrocytes; lymphoproliferative response to Concanavalin A) and the humoral immune response (T cell-dependent IgM antibody formation after injection of sheep erythrocytes and T cell-independent IgM antibody formation after injection of sheep erythrocytes coated with lipopolysaccharides) were dose-dependent and biphasic with initial stimulation and subsequent suppression. The observed biphasic courses of the immune response are in accordance with the histopathological findings in the lymphoid organs. In the course of the study, the initial increase in lymph follicle activity was followed by a reduction in cell numbers that progressed until cell depletion was evident in the thymus, spleen and lymph nodes. Effects on the phagocytic activity of peritoneal macrophages or on the lymphocyte reaction after administration of mitomycin C in vitro were not detected (see Table 4) (Meera et al. 1992). The histopathological effects were not attributed to a specific dose; in addition, how many of the animals were affected was not reported. The authors noted that the effects were dependent on the time and dose.

**Tab. 4** Immunotoxic effects in Swiss mice after oral administration of lindane with the diet (Meera et al. 1992)

| Dose [mg/kg body weight and day] | 4 weeks | 8 weeks | 12 weeks | 24 weeks |
|---|---|---|---|---|
| no allocation to a dose | activation of lymph nodes, reduction in cell numbers that progressed until cell depletion in the thymus, spleen and lymph nodes | not investigated | lymph nodes: as in the controls<br>thymus: cortical lymphocytes ↓, medulla: some necrotic cells<br>spleen: lymph follicles ↓ | lymph nodes: loss of demarcation between cortex and paracortex<br>thymus: loss of demarcation between cortex and medulla<br>spleen: cell depletion in red and white pulp |
| 0.012 and above: dose-dependent, LOAEL | DTH: stimulation of the immune response ↑ (40% at the most),<br>LP: ↑,<br>IgM plaque production ↑ | DTH: stimulation of the immune response ↑,<br>LP: maximum proliferation,<br>IgM plaque production ↑ | DTH: stimulation of the immune response ↓,<br>LP: control value,<br>IgM plaque production ↓ | DTH: stimulation of the immune response ↓,<br>LP: ↓,<br>IgM plaque production ↓ |

DTH: delayed type hypersensitivity reaction (response to sheep erythrocytes); IgM: immunoglobulin M; LP: lymphoproliferative response to Concanavalin A administration

Male **Hissar mice** were given lindane in doses of 0, 10, 30 or 50 mg/kg diet (about 2, 6, 10 mg/kg body weight and day; conversion factor: 0.2 according to EFSA (2012)) for up to 12 weeks. The primary humoral antibody response to sheep erythrocytes was impaired in animals given a lindane dose of 50 mg/kg diet. At a dose of 30 mg/kg diet and above, the level of the secondary immune response was reduced. No effects on the antibody response were observed at a lindane dose of 10 mg/kg diet (about 2 mg/kg body weight and day) (see Table 5). Survival, feed consumption, body weights and the spleen and thymus weights were not affected. The liver weights were significantly increased in the high dose group in week 6 and thereafter and in the middle dose group after 12 weeks (Banerjee et al. 1996).

**Tab. 5** Immunotoxic effects in Hissar mice after oral administration of lindane with the diet (Banerjee et al. 1996)

| Dose [mg/kg body weight and day] | 8 weeks | 12 weeks |
|---|---|---|
| 2, NOAEL | | |
| 6 | plaque-forming cells ↓ | secondary antibody response ↓ |
| 10 | no effects on primary antibodies, thymus or spleen weights, secondary antibody response ↓ (after 3 and 6 weeks), plaque-forming cells ↓ | primary antibody response ↓ |

Groups of 8 male and 8 female **CD-1 mice** were given lindane (purity: 99.78%) with the diet for 39 weeks in doses of 0, 10, 40 or 160 mg/kg diet (about 1.5, 6 or 24 mg/kg body weight and day; conversion factor: 0.15 according to EFSA (2012)). A positive control group was given a single intraperitoneal dose of cyclophosphamide of 50 mg/kg body weight 2 days before blood sampling. The lymphocyte populations were identified based on the presence of antibodies to CD3, CD4 and CD8 for T lymphocytes, CD19 for B lymphocytes and DX5 for natural killer (NK) cells. The lymphocyte counts were higher in male mice than in female mice (76%) because of the higher counts of CD19-positive B lymphocytes (125% of the control value). The number of NK cells significantly increased by 55% ($p < 0.05$) in the females of the high dose group. The other parameters were not affected (JMPR 2002 a).

Groups of 20 adult **BALB/c mice** were given lindane in doses of 0 or 150 mg/kg diet (about 30 mg/kg body weight and day; conversion factor: 0.2 according to EFSA (2012)) for 1 month. After subsequent immunization with a single intraperitoneal injection of 0.5 ml of a 2% suspension of sheep erythrocytes, no significant difference in the IgM production was detected between the two groups. Treatment with lindane and 4 intragastric doses of $4 \times 10^9$ sheep erythrocytes did not cause changes in the IgA, IgG1, IgG2a or IgG3 titres. However, the IgG2b titre was significantly increased. In the animals treated with lindane at a dose level of 150 mg/kg diet and subsequent oral administration of 1000 Giardia muris cysts, the Giardia infection 28 days later was more persistent and more severe than in the animals not treated with lindane. Giardia trophozoites formed in the small intestine; increased levels of specific antibodies were found (André et al. 1983).

### *Rabbit*

Groups of 6 male **rabbits** (no other details) were given lindane orally in gelatine capsules in doses of 1.5, 3, 6 or 12 mg/kg body weight and day on 5 days a week for 6 weeks. The control animals received oil in gelatine capsules. Once a week, the animals were given an intravenous injection of Salmonella typhimurium $Ty_3$. In the animals treated with lindane, the antibody titres were reduced in a dose-dependent manner (Dési et al. 1978).

## Summary

After the exposure of various species to lindane by inhalation or oral administration, a number of studies reported effects on the thymus, spleen and lymph nodes. Following initial stimulation of the humoral and cell-mediated immune response for up to about 8 weeks of the exposure period, an increased inhibition of the immune response became evident beginning in about week 12 of exposure with a reduction in cell numbers that progressed to cell depletion in the thymus, spleen and lymph nodes. The mechanism involved might have been apoptosis of the T and B lymphocytes induced by activation. The dose level at which this effect is observed depends on the species, animal strain and the end point being investigated. After inhalation exposure, the NOAECs for effects on the spleen and thymus were 0.6 mg/m$^3$ for Wistar rats and 1 mg/m$^3$ for CD-1 mice. After oral exposure, the NOAELs for immunotoxic and immunomodulating effects are 2 mg/kg body weight and day for Hissar mice and 0.45 mg/kg body weight and day for Wistar rats. It remains unclear why histopathological effects on the spleen, thymus and lymph nodes were observed in Swiss mice in the very low dose range of 0.012 mg/kg body weight and day and why positive results were obtained in studies in vitro that investigated cell-mediated and humoral immune responses (Meera et al. 1992). The histopathological effects were not attributed to a specific dose; in addition, how many of the animals were affected was not reported. On this basis, it is difficult to evaluate the immunological effects of lindane in the low dose range.

## Allergenic effects

There are no new data available.

## Reproductive and developmental toxicity

### Fertility

The generation studies carried out with lindane and the data that were published for fertility since the 1998 documentation (Greim 2001) are shown in Table 6.

**Tab. 6** Generation studies with lindane that investigated fertility

| Species, strain, number per group | Exposure | Findings | References |
|---|---|---|---|
| **rat**,<br>Crj:CD(SD)IGS,<br>no data,<br>according to the test guideline at least 20 ♂ and 20 ♀ | **2-generation study,**<br>**OECD Test Guideline 416,**<br>**10 weeks before mating up to 3 weeks after weaning**,<br>0, 10, 60, 300 mg/kg diet (F0 ♀: before mating: 0, 0.6, 3.8, 19.0 mg/kg body weight and day; gestation: 0, 0.6, 3.4, 16.6 mg/kg body weight and day; lactation: 0, 1.5, 8.9, 45.2 mg/kg body weight and day; F1 ♀ before mating: 0, 0.8, 4.9, 25.2 mg/kg body weight and day; gestation: 0, 0.6, 3.6, 18.0 mg/kg body weight and day; lactation: 0, 1.5, 8.7, 41.7 mg/kg body weight and day),<br>purity: 99.5% | **no NOAEL parental toxicity;**<br>**0.6 mg/kg body weight and above**:<br>♂ and ♀ F0, F1 adults: liver: activity of hepatic metabolizing enzymes ↑;<br>♂ F0, F1 adults:<br>kidneys: basophilic tubules, hyaline droplets,<br>**3.4 mg/kg body weight**: **NOAEL perinatal toxicity**;<br>**3.4 mg/kg body weight and above**:<br>F0, F1 adults:<br>liver: centrilobular hypertrophy;<br>♂ F1 adults: thyroid: hypertrophy of the follicular epithelium;<br>**16.6 mg/kg body weight**:<br>F0, F1 adults: body weight gains and feed consumption ↓;<br>♀ mortality (pulmonary congestion, oedema);<br>♀ F0 adults: thyroid: hypertrophy of the follicular epithelium;<br>♂ and ♀ F1 offspring: body weights ↓ (PND 0, 4), delay in sexual maturation;<br>pregnant F1: perinatal convulsions and irritability;<br>F2 offspring: survival index ↓ (PND 0–4 and later, primary cause: nursing abnormalities in dams);<br>**16.6 mg/kg body weight**:<br>**NOAEL fertility**;<br>**NOAEL behavioural toxicity**;<br>no substance-induced effects on: endocrine system or reproduction, AGD, nipple development, oestrus cycle, spermatogenesis, open field test (emotionality), rotarod test (motor coordination), pole-climbing test (learning and memory) | Matsuura et al. 2005; Yamasaki et al. 2005 |
| **rat**,<br>Charles River CD,<br>10 ♂ and 10 ♀ | **3-generation study, 60 days before mating up to 3 weeks after weaning**,<br>0, 25, 50, 100 mg/kg diet (0, 7, 14, 28 mg/kg body weight and day),<br>purity: no data, at least 99%,<br>examination: birth, PND 21 | **14 mg/kg body weight**: **NOAEL parental toxicity**;<br>**28 mg/kg body weight**: **NOAEL fertility, perinatal toxicity**;<br>**28 mg/kg body weight**: offspring of generation 3: liver weights ↑ (PND 21, adjusted for body weights by covariance analysis, ♂: 7, 14, 28 mg/kg body weight: +7%, +10%, +24%***; ♀: 7, 14, 28 mg/kg body weight: +13%*, +13%*, +25%***), no fertility disorders, no effects on litter size, pup weights, lactation, incidence of malformations, development | Greim 2001; Palmer et al. 1978 b |

**Tab. 6** *(continued)*

| Species, strain, number per group | Exposure | Findings | References |
|---|---|---|---|
| **rat**,<br>Charles River CD,<br>6 ♂ and 6 ♀ | **range-finding study, 15 days before mating up to PND 4**,<br>0, 20, 100, 200, 400 mg/kg diet (0, 1.5, 7.4, 14.8, 29.6 mg/kg body weight and day),<br>purity: 99.67% | **1.5 mg/kg body weight**: **NOAEL perinatal toxicity**;<br>**7.4 mg/kg body weight**: **NOAEL parental toxicity**;<br>**7.4 mg/kg body weight and above**: F1: body weights ↓ (PND 1–4; 10–23%);<br>**14.8 mg/kg body weight and above**: F0: body weight gains and feed consumption ↓, number of implantations ↓;<br>**29.6 mg/kg body weight**: **NOAEL fertility**;<br>♀ F0: mortality; number of live pups ↓, ♀/♂ ratio of offspring ↑;<br>no substance-induced effects on: oestrus, mating, fertility, conception, length of gestation;<br>unpublished study, original study report not available | JMPR 2002 a |
| **rat**,<br>Charles River CD,<br>30 ♂ and 30 ♀ | **2-generation study, 10 weeks before mating up to 3 weeks after weaning**,<br>0, 1, 20, 150 mg/kg diet (0, 0.09, 1.7, 13.1 mg/kg body weight and day),<br>purity: 99.5% | **1.7 mg/kg body weight**: **NOAEL perinatal toxicity, parental toxicity**;<br>**13.1 mg/kg body weight: NOAEL fertility**;<br>♀ F0: body weight gains during gestation ↓,<br>♂ and ♀ F0 and F1: periacinar hepatocellular hypertrophy, ♂ F0: pale kidneys, ♂ F1: hydronephrosis, F1 and F2 offspring: body weights ↓, survival index ↓, F2 offspring: delay in development (tooth eruption, hair growth);<br>no substance-induced effects: length of gestation, post-implantation index, mating behaviour, fertility index, number of live offspring, ♀ animals of all generations: no gross-pathological or histopathological changes;<br>unpublished study, original study report not available | ATSDR 2005;<br>JMPR 2002 a |
| **mouse**,<br>CD-1,<br>12 ♀ | **15 days before mating up to PND 21**,<br>0, 1, 3 mg/kg body weight and day,<br>gavage,<br>vehicle: no data,<br>purity: 99.78% | **1 mg/kg body weight**: **NOAEL parental toxicity**;<br>**3 mg/kg body weight**: **NOAEL perinatal toxicity, fertility**;<br>**3 mg/kg body weight:** 2 animals sacrificed in extremis;<br>no effects on reproductive parameters, litter size, survival of the offspring, body weights, clinical signs;<br>unpublished study, original study report not available | JMPR 2002 a |

AGD: anogenital distance; PND: postnatal day; *$p < 0.05$; ***$p < 0.001$

A 2-generation study carried out according to OECD Test Guideline 416 in Crj:CD(SD)IGS rats given lindane with the diet reported increases in the activities of hepatic metabolizing enzymes in the adults of the F0 and F1 generations and increases in basophilic tubules and hyaline droplets in the kidneys of the males at the low dose of 0.6 mg/kg body weight and day and above. At 16.6 mg/kg body weight and day, body weights were reduced in the F1 offspring and survival indices were reduced in the F2 offspring. No substance-induced effects on the endocrine system or on reproduction were observed; tests for behavioural toxicity revealed no unusual findings up to the highest dose tested of 16.6 mg/kg body weight and day (Matsuura et al. 2005; Yamasaki et al. 2005). It was not possible to derive a NOAEL for parental toxicity. The NOAEL for perinatal toxicity was 3.4 mg/kg body weight and day and the NOAEL for fertility was the highest dose tested of 16.6 mg/kg body weight and day.

In a 3-generation study in Charles River CD rats given lindane with the diet, the liver weights were increased by more than 20% in male and female rats of the third generation at the dose level of 28 mg/kg body weight and day (Greim 2001; Palmer et al. 1978 b). The NOAEL for perinatal toxicity and fertility was 28 mg/kg body weight and day, which was the highest dose tested. The NOAEL for parental toxicity was 14 mg/kg body weight and day.

In a range-finding study and a subsequent 2-generation study in rats that have not been published and for which the original study reports are not available, the NOAELs for fertility were the highest doses tested of 13.1 and 29.6 mg/kg body weight and day, respectively. The NOAELs for perinatal toxicity were 1.5 and 1.7 mg/kg body weight and day, respectively, and the NOAELs for parental toxicity were 7.4 and 13.1 mg/kg body weight and day, respectively (ATSDR 2005; JMPR 2002 a). In another unpublished study for which the original study report is not available and in which mice were given lindane before mating, the NOAEL for perinatal toxicity and fertility was 3 mg/kg body weight and

day and the NOAEL for parental toxicity was 1 mg/kg body weight and day (JMPR 2002 a). The data are in line with those obtained by other studies.

Long-term exposure to lindane may cause endocrine disruption in mammals. Treatment with 1 to 40 mg/kg body weight and day disrupted testicular morphology, reduced spermatogenesis and plasma androgen concentrations, inhibited testicular steroidogenesis and reduced reproductive performances in males (no other details; Pagès et al. 2002). Treatment of adult Wistar rats with a single dose of 5 mg/kg body weight led to changes in the activities of enzymes that are involved in steroidogenesis (Saradha et al. 2008). In addition, the number of apoptotic cells in the testes was increased at this dose level (Saradha et al. 2009). When mice were given 3 daily oral doses of 25 mg/kg body weight and day either before or immediately after mating, morphological changes of two-cell embryos and morulae were observed. These effects were not induced at 15 mg/kg body weight and day (Scascitelli and Pacchierotti 2003).

## Developmental toxicity

### Rat

In a prenatal developmental toxicity study in CFY rats that were given gavage doses of lindane from days 6 to 15 of gestation, the average percentage of litters with $14^{th}$ ribs was increased and maternal toxicity was concurrently observed at 20 mg/kg body weight and day (Greim 2001; Palmer et al. 1978 a). These findings were not observed in other studies. The NOAEL for developmental toxicity was 10 mg/kg body weight and day.

A study in Wistar rats given gavage doses of a 50% formulation of lindane from days 6 to 15 of gestation reported no toxic effects on development up to a dose of 25 mg lindane/kg body weight and day (Greim 2001; Khera et al. 1979); thus, the results are consistent with those of the study described above (Greim 2001; Palmer et al. 1978 a).

In Wistar rats, the parameters of locomotor activity were recorded; the distance covered by the offspring increased at doses of 0.125 mg/kg body weight and day and above. Other parameters of this end point were not affected (Johri et al. 2007). As the results for motor activity varied greatly (Makris et al. 2009), the tests require proper validation and positive control data are necessary (Graham et al. 2012). It is not clear from the publication of Johri et al. (2007) whether these two points were taken into account. In addition, it is recommended that the locomotor activity in rats is examined on postnatal days 13, 17 and 21 to assess the ontogeny of habituation, the development of the co-ordination of motor activity and the presence or absence of the characteristic periods of early development. An additional examination on postnatal day 60 serves to identify possible latent changes in motor activity and the persistence of previously observed changes (Graham et al. 2012). In the study of Johri et al. (2007), motor activity was tested at 3, 6 and 9 weeks of age, that is on postnatal days 21, 42 and 63, but early motor development was not examined. The efficacy of classifying the animal groups according to different control groups per dose is questionable. Thus, it is unclear whether the same group was always used as the control; this would lead to habituation in repeated tests. The results for motor activity were not dose-dependent or consistent. Furthermore, exposure was not determined. This study has not been included in the evaluation because of the underlying methodological inadequacies.

Inconsistencies are evident in the discussions of ATSDR (2005) and JMPR (2002 b) of an unpublished study in Han Wistar rats and as the original study is not available, the study has not been included in the evaluation.

A study in Sprague Dawley rats with subcutaneous injection of lindane (CIEL 1976 b) is consistent with the other studies in rats.

A study in rats with intraperitoneal injection from days 12 to 17 of gestation (Brannen et al. 1998) has not been included in the evaluation because of the direct effects of intraperitoneal injection on the foetuses.

Likewise, studies with only single exposure (for example Hassoun and Stohs 1996) have not been included.

### Rabbit

When New Zealand White rabbits were exposed to lindane in a prenatal developmental toxicity study from days 6 to 18 of gestation, the incidences of 13$^{th}$ ribs significantly increased at the highest dose tested of 20 mg/kg body weight and day. Other toxic effects on development were not observed (Greim 2001; Palmer et al. 1978 a). The NOAEL for developmental toxicity was 10 mg/kg body weight and day.

Ultrastructural changes in the spermatozoa were observed in the male offspring of Grimaud hybrid rabbits given an oral lindane dose of 1 mg/kg body weight and day from day 8 of gestation up to the end of lactation (Fausto et al. 2001). Without other parameters of developmental toxicity, structural sperm changes alone are not regarded as sufficient evidence of developmental toxicity, particularly as this is not a standard parameter for the assessment of developmental toxicity.

The results from a study in rabbits with subcutaneous injection of lindane (CIEL 1976 a) are consistent with those from the study of Palmer et al. (1978 a) (Greim 2001).

### Mouse

A study in NMRI mice given gavage doses of lindane from days 11 to 13 or from days 6 to 15 of gestation is available only as an abstract (CIEL 1972). Therefore, NOAELs cannot be derived.

In 2 studies in CD-1 mice with prenatal administration from days 9 to 16 of gestation, reversible effects on the testes and sperm were observed in the male offspring at 15 mg/kg body weight and day and above (Di Consiglio et al. 2009; Traina et al. 2003). Two studies tested only one dose (Di Consiglio et al. 2009; Maranghi et al. 2007); therefore, the dose–response relationship could not be evaluated.

In another study in CD-1 mice given gavage doses of lindane from days 8.5 to 11.5 of gestation, decreases in the number of primordial germ cells per embryo were observed at 15 mg/kg body weight and day and above; according to the authors, these were pro-apoptotic effects (La Sala et al. 2009).

The results from a study in NMRI mice given lindane doses of 6 mg/kg body weight and day from days 6 to 15 of gestation by subcutaneous injection (WHO 1991) are consistent with those from the other studies in mice.

### Dog

Stillbirths were increased in beagle dogs given oral lindane doses of 7.5 or 15 mg/kg body weight and day from day 1 or 5 up to the end of gestation; this effect was not dependent on the dose. Teratogenicity was not observed (Greim 2001).

The studies with prenatal exposure to lindane are shown in Table 7.

**Tab. 7** Studies with prenatal exposure to lindane

| Species | Exposure | Findings | References |
|---|---|---|---|
| **rat** | | | |
| CFY,<br>20 ♀ | **GD 6–15**,<br>similar to OECD Test Guideline 414, sufficiently valid for the year in which the study was conducted,<br>0, 5, 10, 20 mg/kg body weight and day,<br>gavage,<br>vehicle: 0.5% aqueous carboxymethyl cellulose,<br>purity: no data,<br>examination: GD 20 | **10 mg/kg body weight**: **NOAEL developmental toxicity**,<br>**10 mg/kg body weight and above**: dams: body weight gains ↓;<br>**20 mg/kg body weight**: dams: 2 died, foetuses: incidence of 14th ribs ↑ (dose-dependent, total number at 0, 5, 10, 20 mg/kg body weight: 17, 29, 49, 54; average percentage per litter: 12.7%, 21.0%, 31.7%, 40.6%; statistically significant increase in percentage per litter only at the highest dose);<br>not reproduced in other studies | Greim 2001 |
| Wistar,<br>20 ♀ | **GD 6–15**,<br>0, 6.25, 12.5, 25 mg/kg body weight and day,<br>gavage,<br>vehicle: no data,<br>purity: formulation with 50% lindane or hexachlorobenzene, remaining substances of the formulation unknown,<br>examination: GD 22 | **25 mg/kg body weight**: **NOAEL developmental toxicity**,<br>**25 mg/kg body weight**: dams: slight decrease in body weights | Greim 2001 |
| Wistar,<br>32 ♀ | **GD 5–21**,<br>0, 0.0625, 0.125, 0.25 mg/kg body weight and day,<br>oral,<br>vehicle: corn oil,<br>purity: technical grade,<br>examination: locomotor activity of the offspring at 3, 6, 9 weeks of age (8 animals in each case) | **0.0625 mg/kg body weight and above**: offspring: liver, brain: mRNA and protein expression ↑ (CYP1A1, 1A2, 2B1, 2B2);<br>**0.125 mg/kg body weight and above**: offspring: changes in locomotor activity (distance covered ↑: after 3 weeks, 0.25 mg/kg body weight: after 3, 6, 9 weeks);<br>**0.25 mg/kg body weight**: offspring: changes in locomotor activity (resting period ↓ after 3, 6 weeks), earlier onset and increased incidence of convulsions after a single sub-convulsive dose of lindane of 30 mg/kg body weight;<br>not included in the evaluation because of underlying methodological inadequacies | Johri et al. 2007, 2008 b, a |
| Han Wistar,<br>24 ♀ | **GD 6–PND 10**,<br>0, 10, 50, 120 mg/kg diet,<br>gestation: 0, 0.8–0.9, 4.2–4.6, 8–10 mg/kg body weight and day,<br>lactation: 0, 1.2–1.7, 5.6–8.3, 14–19 mg/kg body weight and day,<br>purity: 99.78%,<br>examination: F1 offspring per sex: FOB: PND 4, 11, 21, 35, 45, 60; motor activity: PND 13, 17, 22, 59; startle response: PND 28, 60, tests for learning and memory: water maze test: PND 28, 65 | **4.2 mg/kg body weight**: **NOAEL perinatal toxicity**;<br>**4.2 mg/kg body weight**: offspring: motor activity ↑, ♀: habituation of motor activity ↓;<br>**8.0 mg/kg body weight**: dams: body weight gains and feed consumption ↓, sensitivity to touch ↑; offspring: body weights and body weight gains ↓ (PND 1–11), auditory startle response habituation ↓, number of stillbirths ↑, survival index ↓;<br>unpublished study, original study not available,<br>according to US EPA, study cannot be used to evaluate behaviour because the behavioural tests were not validated and the sample size was too small with 6 animals per group (JMPR 2002 b); inconsistent data in ATSDR and JMPR, for example for the number of animals examined (6 and 10, respectively), day of examination of the motor activity (PND 11, 60 and 13, 17, 22, 59, respectively)<br>study has not been included in the evaluation | ATSDR 2005;<br>JMPR 2002 b |
| Sprague Dawley,<br>20 ♀ | **GD 6–15**,<br>0, 5, 15, 30 mg/kg subcutaneous,<br>vehicle: no data,<br>purity: no data,<br>examination: no data | **15 mg/kg body weight and above**: dams: body weight gains ↓;<br>**30 mg/kg body weight**: dams: 2 died,<br>number of live foetuses unchanged, no skeletal or visceral anomalies | Greim 2001 |

**Tab. 7** *(continued)*

| Species | Exposure | Findings | References |
|---|---|---|---|
| **rabbit** | | | |
| New Zealand White, 20–24 ♀ | **GD 6–18**, similar to OECD Test Guideline 414, sufficiently valid for the year in which the study was conducted, 0, 5, 10, 20 mg/kg body weight and day, gavage, vehicle: 0.5% aqueous carboxymethyl cellulose, purity: no data, examination: GD 29 | **no NOAEL maternal toxicity**; **5 mg/kg body weight and above**: dams: lethargy, tachypnoea, body weight gains ↓; **10 mg/kg body weight**: **NOAEL developmental toxicity**; **20 mg/kg body weight**: dams: pre-implantation losses ↑; foetuses: incidence of 13$^{th}$ ribs ↑ (average percentage per litter: 0, 5, 10, 20 mg/kg body weight: 63 %, 42 %, 53 %, 85 %; statistically significant increase only in the percentage per litter at the highest dose, no data for the total number) | Greim 2001 |
| Grimaud hybrid, 15 ♀ | **gestation and lactation (beginning: 8 days after insemination, daily for 2 weeks, then every 2nd day up to the end of lactation)**, 0, 1 mg/kg body weight and day, oral by syringe, vehicle: corn oil, purity: no data, examination: PND 194, 215 and 236 | **1 mg/kg body weight**: ♂ offspring: spermatozoa: ultrastructural changes (cytoplasmic inclusions, coiled tails), not a standard parameter of developmental toxicity | Fausto et al. 2001 |
| strain not given, 15 ♀ | **GD 6–18**, 0, 5, 15, 45 mg/kg body weight and day, subcutaneous, vehicle: no data, purity: no data, examination: no data | **15 mg/kg body weight and above**: dams: body weight gains ↓; **45 mg/kg body weight**: dams: severe toxicity, therefore dose reduced to 30 mg/kg body weight, nevertheless 14/15 rabbits died; no embryotoxic or teratogenic effects | Greim 2001 |
| **mouse** | | | |
| NMRI, 25 ♀ | **GD 11–13 or GD 6–15**, 0, 12, 30, 60 mg/kg body weight and day, gavage, vehicle: no data, purity: no data, examination: no data | GD 6–15: **30 mg/kg body weight**: dams: 1 died, malformations: 4.2% (regarded as spontaneous); **60 mg/kg body weight**: dams: 12 died, body weight gains ↓, foetuses: body weights ↓, miscarriages ↑, number of foetuses ↓, malformations: 0%; GD 11–13: **60 mg/kg body weight**: dams: 4 died; study available only as an abstract | Greim 2001 |
| CD-1, 10 ♀ | **GD 9–16**, 0, 25 mg/kg body weight and day, gavage, vehicle: olive oil, purity: technical grade, examination: PND 50, 65–69, 100 | **25 mg/kg body weight**: ♂ offspring: sperm concentrations ↓ (PND 65–69, PND 100: completely recovered), activity of the testosterone 6beta-hydroxylase, 2alpha-hydroxylase and dehydrogenase ↓ (PND 65–69, PND 100: almost recovered); no systemic toxicity in the offspring, no mortality or effects on body weights in dams or offspring | Di Consiglio et al. 2009 |
| CD-1, 12 ♀ | **GD 9–16**, 0, 15 mg/kg body weight and day, gavage, vehicle: olive oil, purity: technical grade, examination: PND 22 | **15 mg/kg body weight**: ♀ offspring: absolute and relative uterus weights ↑, earlier vaginal opening, diameter of primary oocytes ↓; no effects on steroid hormone metabolism; from in vitro studies: ER-beta-mediated effects | Maranghi et al. 2007 |

**Tab. 7** *(continued)*

| Species | Exposure | Findings | References |
|---|---|---|---|
| CD-1<br>10 ♂ or 12 ♀ | **GD 9–16**,<br>0, 15, 25 mg/kg body weight and day,<br>positive control: 10 µg diethylstilbestrol/kg body weight and day,<br>gavage,<br>vehicle: olive oil,<br>purity: technical grade,<br>examination: PND 60, 100 | **15 mg/kg body weight and above**: ♂ offspring: testis: number and size of Leydig cells ↑ (PND 60, not on PND 100), number of sperm head/testis ↓ (PND 60, not on PND 100), epididymis: number of sperm with chromatin abnormalities (PND 60, not on PND 100) ↑;<br>**25 mg/kg body weight**: ♂ offspring: testis: changes in germ cell distribution (PND 60, 100), sperm head count/g testis) ↓ (PND 60, 100) | Traina et al. 2003 |
| CD-1,<br>♀, number not given | **GD 8.5–11.5**,<br>0, 15, 30 mg/kg body weight and day,<br>gavage,<br>vehicle: olive oil,<br>purity: technical grade,<br>examination: GD 12.5 | **15 mg/kg body weight and above**: embryos: number of primordial germ cells/embryo ↓ | La Sala et al. 2009 |
| NMRI,<br>25 ♀ | **GD 11–13 or GD 6–15**,<br>0, 6 mg/kg body weight and day,<br>subcutaneous,<br>vehicle: no data,<br>purity: no data,<br>examination: no data | no maternal toxicity or developmental toxicity, no increased incidence of malformations | Greim 2001 |
| **dog** | | | |
| beagle,<br>13 or 14 ♀ | **GD 1 or GD 5–end of gestation**,<br>0, 7.5, 15 mg/kg body weight and day,<br>oral,<br>purity: no data,<br>examination: no data | stillbirths ↑ independent of the dose, number of live pups unchanged compared with the control group, no teratogenicity | Greim 2001 |

ER: oestrogen receptor; FOB: functional observational battery; GD: gestation day; PND: postnatal day

## Summary

Prenatal oral exposure to lindane did not induce teratogenicity in rats up to doses of 25 mg/kg body weight and day (Greim 2001; Khera et al. 1979), in rabbits up to doses of 20 mg/kg body weight and day (Greim 2001; Palmer et al. 1978 a) and in dogs up to doses of 15 mg/kg body weight and day (WHO 1991). In a prenatal developmental toxicity study in CFY rats, increases in the incidence of 14$^{th}$ ribs with concurrent maternal toxicity in the form of reduced body weight gains were observed at the dose of 20 mg/kg body weight and day (Greim 2001; Palmer et al. 1978 a). The NOAEL for developmental toxicity was 10 mg/kg body weight and day. In a prenatal developmental toxicity study in New Zealand White rabbits, the incidence of 13$^{th}$ ribs was increased at the dose of 20 mg/kg body weight and day. Lethargy, tachypnoea and reduced body weight gains were observed in the dams at the lowest dose tested of 5 mg/kg body weight and day and above (Greim 2001; Palmer et al. 1978 a). The NOAEL for developmental toxicity was also 10 mg/kg body weight and day. In a 2-generation study carried out according to OECD Test Guideline 416 in Crj:CD(SD)IGS rats given lindane with the diet, the body weights of the F1 offspring and the survival indices of the F2 offspring were reduced at the dose of 16.6 mg/kg body weight and day. Increases in the activities of hepatic metabolizing enzymes in the adults of the F0 and F1 generations and increases in basophilic tubules and hyaline droplets in the kidneys of the males were observed at the lowest dose tested of 0.6 mg/kg body weight and day and above. Tests for behavioural toxicity in the offspring revealed no unusual findings up to the highest dose tested of 16.6 mg/kg body weight and day. The NOAEL for perinatal toxicity was 3.4 mg/kg body weight and day (Matsuura et al. 2005; Yamasaki et al. 2005).

## Genotoxicity

Documentation for lindane was published in 1998 (Greim 2001), followed by a supplement for genotoxicity in 2002 (Greim 2002). The findings relating to the genotoxicity induced by lindane are summarized on the basis of these publications and supplemented by the results that have been published since 2001.

### In vitro

Lindane did not have DNA-damaging or mutagenic effects in bacterial test systems. In mammalian cells, no sister chromatid exchanges, DNA repair synthesis, chromosomal aberrations or gene mutations were observed (Greim 2001, 2002).

As described in detail in the 1998 documentation (Greim 2001) and in the 2002 supplement (Greim 2002), lindane induced DNA strand breaks in several types of cells. This was confirmed by the findings of an alkaline comet assay, which reported dose-dependent increases in DNA fragments in human epithelial cells of the nasal conchae and tonsils after incubation with lindane (0.5, 0.75 and 1.0 mM) for 60 minutes (Tisch et al. 2001, 2002, 2005).

In a study in human fibroblasts, lindane (> 6 mM) was co-incubated with hydrogen peroxide (40 µM and 50 µM) and investigated for DNA strand breaks by means of the alkaline comet assay. Co-incubation revealed significant increases in DNA damage compared with incubation with hydrogen peroxide alone. The study did not investigate incubation with lindane only (Lueken et al. 2004).

In addition, DNA damage was detected by the alkaline comet assay in isolated human peripheral lymphocytes at a lindane concentration of 20 µg/ml and above (Nair et al. 2005).

In this study, chromosomal aberrations were observed in isolated human peripheral lymphocytes at a lindane concentration of 10 µg/ml and above after an incubation period of 72 hours (Nair et al. 2005). However, the study was carried out without the addition of a metabolic activation system and values for the cytotoxicity (mitotic index) of the controls were not reported. Furthermore, the data did not show whether the subjects from whom the peripheral lymphocytes were collected had previously been exposed to other pesticides. Therefore, the validity of the study is questionable.

No DNA fragments were found, however, in human peripheral blood cells after incubation with lindane for 24 hours at a concentration of 20 mg/l (Bharathi et al. 2013). Likewise, the number of DNA strand breaks was not increased in a study that investigated DNA strand breaks in primary rat hepatocytes by alkaline elution with 0.08 and 0.12 mM lindane (Gealy et al. 2007).

In a study that tested low lindane concentrations of $10^{-12}$ M, $2\times10^{-12}$ M, $10^{-11}$ M, $2\times10^{-11}$ M and $5\times10^{-11}$ M in the human MCF-7 breast cancer cell line for 24 hours without the addition of a metabolic activation system, 32, 47, 57, 54 and 48 micronuclei were observed per 1000 binucleate cells. Similar effects were induced in cells of the human prostate cell line PC-3 (Kalantzi et al. 2004).

The incubation of cells of the human MCF-7 breast cancer cell line with low lindane concentrations ($10^{-12}$ M, $10^{-11}$ M and $10^{-10}$ M) for 24 hours without metabolic activation revealed increases of 67, 64 and 78 micronuclei in 1000 binucleate cells. In the DMSO control, 21 micronuclei were counted in 1000 binucleate cells. Furthermore, the addition of lindane induced 20%, 30% and 45% decreases in the mitotic rates depending on the concentration (Hewitt et al. 2007).

### In vivo

No positive findings were induced by lindane in vivo in the studies for chromosomal aberrations or micronuclei that were listed in the 1998 documentation (Greim 2001) or in the 2002 supplement (Greim 2002).

Data that were published after 2002 are described below.

Lindane was administered by intraperitoneal injection to groups of 6 male Park mice in doses of 0, 35 or 70 mg/kg body weight. After 24 hours, 1000 polychromatic cells from the bone marrow of each animal were scored. The number of erythrocytes with micronuclei per 1000 polychromatic erythrocytes increased with the dose and amounted to 24 (57.5%) and 34 (70.2%). The mean percentages of polychromatic erythrocytes with micronuclei were 0.4 ± 0.04 and 0.57 ± 0.08;

compared with the values for the controls of 0.17 ± 0.06, the increases were statistically significant ($p < 0.05$). The mean percentages of the total number of polychromatic erythrocytes within the specific groups (32.85 ± 0.7 and 27.67 ± 0.44) decreased significantly ($p < 0.0001$) compared with the value for the controls (43.30 ± 0.58). This is a sign of cytotoxicity and regarded as evidence that the bone marrow has been reached. The positive control substance cyclophosphamide induced the expected effects (Yaduvanshi et al. 2012). Clastogenic effects were observed only at cytotoxic doses.

Male Swiss mice (8–10 animals per group) were continuously administered oral lindane doses of 0 or 80 mg/kg body weight, dissolved in olive oil, for 96 hours (no other details regarding administration). From each animal, 100 chromosomes in the metaphase from the bone marrow were examined for chromosomal aberrations and micronuclei were determined in 500 polychromatic erythrocytes. Compared with the incidence in the controls, the 27.3% increase in chromosomal aberrations was not statistically significant. The mitotic index was significantly decreased by 12.3%. Compared with the number in the controls, the 21.6% increase in the percentage of polychromatic erythrocytes with micronuclei was not statistically significant (Nagda and Bhatt 2015). In this study, the doses were reported inconsistently and no further information is provided about the types of aberrations (for example, gaps and breaks). The published figures suggest that the chromosomes were not prepared properly because they overlap each other in the metaphases and are therefore not spread sufficiently. This study has not been included in the evaluation because of its restricted validity.

Male Wistar rats (8 animals per group) were given intraperitoneal injections of lindane in doses of 0 or 300 mg/kg body weight, and the bone marrow of the animals was examined 48 hours after treatment (no other details). As shown by the published figures, the micronucleus frequencies (about 4.5%) were increased in comparison with the incidence in the controls (about 0.5%) (Anilakumar et al. 2009). As the study and its results were not described in sufficient detail, this study has likewise not been included in the evaluation.

### Summary

The in vitro studies demonstrated that lindane causes DNA strand-breaking effects. The induction of oxidative stress by lindane was described in detail for rats and has been confirmed in recent studies after intraperitoneal injection (see also Greim 2001 and Greim 2002).

Contrary to earlier findings, lindane has been found to induce clastogenic effects. Two of three in vivo studies are considered invalid because of methodological inadequacies or insufficient documentation. The third and valid study found that micronuclei were induced in polychromatic erythrocytes of the bone marrow of mice after intraperitoneal injection of lindane doses of 35 or 70 mg/kg body weight, but reported concurrent findings of cytotoxicity (Yaduvanshi et al. 2012). In contrast, valid micronucleus tests carried out in 3 species (NMRI mice, Chinese hamsters and Sprague Dawley rats) yielded negative results. The doses that were tested in NMRI mice were 35, 50 and 70 mg/kg body weight (Greim 2002). Lindane was shown to reach the bone marrow at these concentrations by the cytotoxicity induced in the study of Yaduvanshi et al. (2012). Furthermore, the 2002 supplement (Greim 2002) included a valid dominant lethal test that yielded no evidence of clastogenicity in the germ cells. Lindane was found to have DNA strand-breaking potential, which is very probably induced by oxidative stress and is regarded as an indicator of genotoxicity. However, as a further mutagenicity or clastogenicity is not induced, it is not assumed that lindane is genotoxic in vivo.

## Carcinogenicity

Liver carcinomas were observed in mice given lindane with the diet in doses of 600 mg/kg diet (about 90 mg/kg body weight and day) for 6 months and in mice given lindane in the diet at a dose level of 80 mg/kg diet and above (about 12 mg/kg body weight and day) for 2 years. No carcinomas were found in rats, but neoplastic foci developed. In an initiation–promotion study in rats given the substance with the diet for 20 weeks, lindane was found to have tumour-promoting effects on the liver; liver foci developed in animals given doses of 2.5 mg/kg body weight and day and above (Greim 2001). There are no new studies available for carcinogenicity in animals.

## Other effects

### Immunotoxic effects

In vitro

Thymocytes from male C57BL/6 mice were exposed to 50 to 150 µM lindane for 12 hours. Significant increases in superoxide anions and hydrogen peroxide were observed as early as after exposure for 5 minutes and after another 10 minutes to the low concentration. A mixture of lindane and malathion induced 4-fold increases in superoxide anions and thus supra-additive effects. No changes were observed in the activity of the superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase enzymes after exposure to lindane only. After exposure to mixtures of lindane and malathion or lindane and permethrin, the superoxide dismutase activities and glutathione peroxidase and reductase activities were significantly reduced (Olgun and Misra 2006).

Splenocytes (90% lymphocytes) from male C57BL/6 mice were exposed to lindane concentrations of 100, 150 or 300 µM for 4, 8 and 16 hours. The cytotoxicity increased to about 75% at these concentrations (as taken from a figure). A concentration of 70 µM lindane was extrapolated for further testing. After treatment with 70 µM lindane, cytotoxicity increased linearly with the duration of exposure for 4, 8 and 16 hours. Necrotic processes were observed, such as increased cytoplasm and nuclear swelling. Concurrent nuclear fragmentation indicated apoptosis. Survival was significantly reduced and both early apoptosis and late apoptosis/necrosis were significantly increased (Battaglia et al. 2010).

# Manifesto (MAK value/classification)

Lindane has tumour-promoting potential in the liver of rats and induced liver carcinomas in mice. Immunomodulating effects were demonstrated in mice, rats and rabbits.

**MAK value.** Liver end point: Tumour promotion in the liver can be explained by receptor-mediated, hormone-like effects and the inhibition of apoptosis. In the case of lindane, the induction of cytochrome P450-dependent monooxygenases correlates with the area of the enzyme-modified foci in the liver. A NOAEL can be determined for both processes because the dose–response relationships are not linear. The data currently available indicate that tumour-promoting effects may be avoided at dose levels at which cytochrome P450-dependent monooxygenases are not induced. Enzyme induction is thus regarded as a biomarker, but it is probably not causally related to the tumour-promoting effects. However, enzyme induction is causally related to hypertrophic and hyperplastic effects in the liver; it is assumed that they are at least involved in the tumour-promoting effects of the enzyme-inducing xenobiotic substance. The induction of monooxygenase activities was observed in workers at serum lindane concentrations above 10 µg/l. Reliable data for the concentrations of lindane in air that correlate with these serum concentrations or that would permit the derivation of a NOAEL are not available. Average serum concentrations of 36.9 µg/l were determined in workers exposed to lindane concentrations of 0.004 to 0.15 mg/m$^3$; there was evidence of the induction of monooxygenase activities. However, adverse effects on the health of these workers were not reported. It can be concluded from the description of the exposure conditions that the workers had intensive skin contact with lindane, which contributed considerably to the body burden in these workers. Therefore, the serum concentrations cannot be correlated directly with the concentrations in air. In an inhalation study with rats, the NOEC (no observed effect concentration) for the induction of monooxygenases was 0.6 mg/m$^3$.

Immunotoxicity end point: After exposure to lindane by inhalation or oral administration, the first signs of inhibition of the immune response became evident in about week 12 of exposure with a reduction in cell numbers that progressed to cell depletion in the thymus, spleen and lymph nodes. The mechanism involved might have been the activated apoptosis of the T and B lymphocytes. The dose level at which this effect is observed depends on the species, animal strain and the end point investigated. After long-term inhalation exposure, the NOECs for effects on the spleen and thymus were 0.6 mg/m$^3$ for Wistar rats and 1 mg/m$^3$ for CD-1 mice. After oral exposure, the NOAELs for immunotoxic and immunomodulating effects were 2 mg/kg body weight and day for Hissar mice and 0.45 mg/kg body weight and

day for Wistar rats. It remains unclear why in Swiss mice histopathological effects on the spleen, thymus and lymph nodes were observed in the very low dose range of 0.012 mg/kg body weight and day and why positive results were obtained in studies in vitro that investigated cell-mediated and humoral immune responses (Meera et al. 1992). The histopathological effects were not attributed to the respective doses and the number of animals affected was not reported. On this basis, it is difficult to evaluate the effects in this low dose range.

After chronic inhalation exposure, the NOECs for histopathological effects on the spleen, thymus and bone marrow were 0.6 mg/m$^3$ (rat) or 1 mg/m$^3$ (mouse) and the NOAELs for immunomodulating effects were 0.45 mg/kg body weight and day for rats and 2 mg/kg body weight and day for mice; therefore, the MAK value of 0.1 mg/m$^3$ has been retained. After exposure to lindane at the MAK value of 0.1 mg/m$^3$ and assuming 100% absorption by inhalation, 70 kg body weight, 10 m$^3$ respiratory volume and no direct skin contact, absorption is calculated to be 0.014 mg/kg body weight per day. Exposure to concentrations in such a low range is not expected to inhibit the immune response in humans.

**Peak limitation.** In view of the systemic effects, lindane remains classified in Peak Limitation Category II, and the excursion factor of 8 has been retained because of the long half-life (Greim 2002).

**Prenatal toxicity.** The data for humans are not suitable for the evaluation of the developmental toxicity of lindane, either because the exposure concentrations were not reported or the persons were exposed to several substances.

In a prenatal developmental toxicity study in CFY rats, increases in the incidence of 14$^{th}$ ribs together with maternal toxicity in the form of reduced body weight gains were observed at 20 mg/kg body weight and day (Greim 2001; Palmer et al. 1978 a). The NOAEL for developmental toxicity was 10 mg/kg body weight and day. In a prenatal developmental toxicity study in New Zealand White rabbits, the incidence of 13$^{th}$ ribs was increased at 20 mg/kg body weight and day. Lethargy, tachypnoea and reduced body weight gains were observed in the dams at the lowest dose tested of 5 mg/kg body weight and day and above (Greim 2001; Palmer et al. 1978 a). The NOAEL for developmental toxicity was 10 mg/kg body weight and day. In a 2-generation study carried out according to OECD Test Guideline 416 in Crj:CD(SD)IGS rats given lindane with the diet, the body weights of the F1 offspring and the survival index of the F2 offspring were reduced at 16.6 mg/kg body weight and day. Increases in the activities of metabolizing hepatic enzymes in the adults of the F0 and F1 generations and increases in basophilic tubules and hyaline droplets in the kidneys of the males were observed at the lowest dose tested of 0.6 mg/kg body weight and day and above. Tests for behavioural toxicity in the offspring revealed no unusual findings up to the highest dose tested of 16.6 mg/kg body weight and day (Matsuura et al. 2005). The NOAEL for perinatal toxicity was 3.4 mg/kg body weight and day.

Oral absorption in rats and mice is complete (ATSDR 2005). Therefore, oral absorption is assumed to be 100% in rats and rabbits. The resulting calculated concentrations and margins between the NOAELs and the MAK value of 0.1 mg/m$^3$ are shown in Table 8.

**Tab. 8** NOAELs in rats and rabbits relevant to the evaluation, toxicokinetic extrapolation of the NOAELs to concentrations in air and resulting margins between these values and the MAK value of 0.1 mg/m$^3$

| Species, exposure | NOAEL: end point | Toxicokinetic extrapolation[a] (mg/m$^3$) | Margin between the extrapolated value/NOAEC and the MAK value of 0.1 mg/m$^3$ |
|---|---|---|---|
| **rat** | | | |
| **prenatal, gavage** | 10 mg/kg body weight and day: developmental toxicity | 17.5 | 175 |
| | LOAEL: 20 mg/kg body weight and day | 35 | 350 |
| **prenatal and postnatal, diet** | 3.4 mg/kg body weight: perinatal toxicity | 8.3[b] | 83 |
| | LOAEL: 16.6 mg/kg body weight and day | 40.7[b] | 407 |
| **rabbit** | | | |
| **prenatal, gavage** | 10 mg/kg body weight and day: developmental toxicity | 29 | 290 |
| | LOAEL: 20 mg/kg body weight and day | 58 | 580 |

[a] (1:4 or 1:2.4) × 1.0 (oral absorption in animals)/1.0 (absorption by inhalation in humans)
[b] additional extrapolation of 7-day treatment in animals to 5-day exposure at the workplace

Therefore, the margins between the MAK value and the calculated NAECs for developmental toxicity and perinatal toxicity are sufficiently large, and lindane remains classified in Pregnancy Risk Group C.

**Carcinogenicity.** Liver carcinomas were observed in mice given lindane in a concentration of 600 mg/kg diet (about 90 mg/kg body weight and day) for 6 months and those given lindane in a concentration of 80 mg/kg diet and above (about 12 mg/kg body weight and day) for 2 years. In a chronic study in rats fed lindane with the diet, neoplastic foci were observed in the liver. The incidences of liver carcinomas were not increased. In an initiation-promotion study with rats given lindane with the diet for 20 weeks, lindane was shown to have tumour-promoting effects on the liver; liver foci developed in animals at 2.5 mg/kg body weight and day and above.

The tumour incidences of adenomas and carcinomas of the thyroid that were not significantly increased in the long-term feeding study in rats can be attributed to the induction of glucuronosyltransferases (phase II enzymes) and are evidence of the increased sensitivity of rodents to the induction of thyroid tumours. Tumour incidences were not increased in any other organ.

Lindane has DNA strand-breaking potential, which is very probably induced by oxidative stress. However, a large number of tests did not yield evidence of mutagenicity or clastogenicity; therefore, lindane is not regarded as genotoxic.

Several studies found an increased risk of developing non-Hodgkin's lymphomas among those who handled lindane. The risk increased with the length and intensity of application. The risk decreased if adjustment was made for other pesticides, mainly chlorinated organic pesticides. The exposure levels were not recorded and biomonitoring was not carried out in any of the studies. The authors discussed whether lindane contributes to the development of non-Hodgkin's lymphomas; this requires further research. As animal studies provided evidence that lindane causes immunomodulating effects, an association on the basis of the mechanism of action would be plausible. However, it has to be taken into account that when the findings of the Agricultural Health Study were evaluated according to the type of livestock, also farmers raising poultry and working in a poultry confinement area had a significantly increased risk of developing non-Hodgkin's lymphomas. Due to lacking information on the level of exposure and possible confounders, such as other chlorinated organic pesticides, the data are not sufficient to confirm that lindane induces carcinogenic effects in humans. Lindane remains classified in Carcinogen Category 4 because of the carcinogenic effects in mice, the tumour-promoting effects in rats and the lack of genotoxicity.

**Germ cell mutagenicity.** In 2002 (Greim 2002), lindane was not classified in one of the germ cell mutagen categories because no positive findings were obtained in studies that investigated the induction of gene or chromosomal mutations in vitro or the induction of micronuclei or chromosomal aberrations in somatic cells in vitro or in vivo. In addition, a valid dominant lethal test yielded no evidence of germ cell mutagenicity. The studies that have been published since 2002 support this decision. Lindane is not regarded as genotoxic and therefore classification in one of the germ cell mutagen categories is not required.

**Absorption through the skin.** In an in vivo study, the amount dermally absorbed was estimated to be 8 mg for humans after exposure to a wood preservative containing 0.3% lindane under standard conditions (2000 $cm^2$ surface area of skin and 1-hour exposure). About 1 mg is absorbed in 8 hours after exposure at the MAK value at a respiratory volume of 10 $m^3$ and 100% absorption by inhalation. Therefore, absorption through the skin may exceed absorption by inhalation and the designation with "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) has been retained.

**Sensitization.** No positive clinical findings for the sensitizing effects of lindane on the skin are available in humans and no experimental animal studies were carried out. Likewise, there are no findings available for the sensitizing effects on the respiratory tract. Lindane is therefore not designated with either "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

# Notes

## Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (https://www.dfg.de/en/dfg_profile/statutory_bodies/senate/health_hazards/conflicts_interest/index.html) ensure that the content and conclusions of the publication are strictly science-based.

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