Chlorpyrifos

(CAS No: 2921-88-2)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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1 Introduction

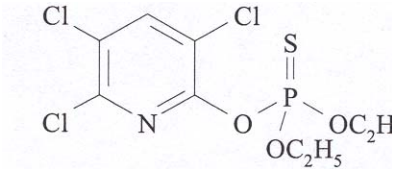
The present document contains the assessment of the health hazard of chlorpyrifos by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by AAE Wibowo, Ph.D. and MM Verberk, Ph.D. (Coronel Institute of the Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands).

The evaluation of the toxicity of chlorpyrifos has been based on the reviews published in the 'Handbook of pesticide toxicology' (Gal91) and by the American Conference of Governmental Industrial Hygienists (ACG99) and Richardson (Ric95), Cochran et al. (Coc95), and Minton and Murray (Min88). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in April 2000, literature was searched in the on-line databases Medline, Embase, and Chemical Abstracts, starting from 1966, 1988 and 1970, respectively, as well as in the CD-ROM versions of the databases Poltox (1990-1994) HSELINE, CISDOC, MHIDAS, and NIOSHTIC (from 1998 backwards), and using the following key words: chlorpyrifos, dursban, and 2921-88-2. The Hazardous Substances Data Bank (HSDB) was also consulted (NLM02). Data of unpublished studies were generally not taken into account. Exceptions were made for studies that were summarised and evaluated by international bodies such as the Food and Agricultural Organization/World Health Organization (FAO/WHO: Joint Meeting of the FAO Panel of Experts on Pesticides Residues on Food and the Environment and the WHO Expert Group on Pesticides Residues - JMPR) (FAO00) and the Health Effects Division (HED) of the US Environmental Protection Agency (EPA) as part of its hazard identification assessment review (EPA00).

In October 2002, the President of the Health Council released a draft of the document for public review. Comments were received from the following individuals and organisations: PA Watson (Dow AgroSciences, Abingdon, England) and J Soave (Health and Safety Executive, London, England). These comments were taken into account in deciding on the final version of the document.

An additional search in Toxline and Medline in April 2003 did not result in information changing the committee's conclusions.

2 Identity

name	:	chlorpyrifos
synonym	:	phosphorothioic acid, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) ester; *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate; Dursban 4E; Detmol U.A.; Lorsban; Dowco 79
molecular formula	:	$C_9H_{11}Cl_3NO_3PS$
structural formula	:	
CAS number	:	2921-88-2

3 Physical and chemical properties

molecular weight	:	350.59
boiling point	:	162ºC (decomposes)
melting point	:	42.5-43ºC
vapour pressure	:	at 25ºC: 2.5 x 10^{-3} Pa
solubility in water	:	practically insoluble (at 25ºC: 0.14 mg/100 mL)
Log $P_{octanol/water}$	:	4.96 (experimental); 4.66 (estimated)
conversion factors	:	not applicable

Data from: ACG99,FAO73, Gal91, NLM03, http://esc.syrres.com.

Pure chlorpyrifos is a colourless to white, crystalline solid with a mild mercaptan odour. Chlorpyrifos is stable under normal storage conditions. The half-life in aqueous methanolic solution at pH 6 is 1930 days; at pH 10, it is 7.2 days (Gal91).

4 Uses

Chlorpyrifos is used to control many types of insect pests in a wide range of crops and ornamentals. It is also used to control mosquitoes, flies, and household pests, including termites (Ric95, Rob99, Sul82). The compound is applied in

emulsifiable concentrates, dust, flowable pellet, spray and granular wettable powder (NLM02).

According to the database of the Dutch Pesticide Authorisation Board (CTB)*, chlorpyrifos is at present registered in the Netherlands for its use as an active ingredient in a few formulations controlling crawling insects (in specific spaces), moths (protecting woollen fabrics), and cabbage maggots (on cabbage varieties).

5 Biotransformation and kinetics

Human data

Six male human volunteers were given an oral dose of chlorpyrifos of 0.5 mg/kg bw. Approximately 70% of the administered dose was excreted in the urine as the metabolite 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), most of it within 24 hours after dosing. Peak 3,5,6-TCP concentrations in the blood were reached after 6 hours. No unchanged chlorpyrifos could be detected. Four weeks later, 5 of the same men received a single dermal dose of 5 mg/kg bw of chlorpyrifos dissolved in dipropylene glycol methyl ether. The amount of the dose excreted in the urine as 3,5,6-TCP within 120 hours after dosing was 1.3%. Peak concentrations of the metabolite in blood were reached 24 hours after dosing. The half-life of elimination of 3,5,6-TCP from the blood and the urine was 27 h following both oral and dermal doses (Nol84). Chlorpyrifos metabolites diethyl phosphate (DEP) and diethyl phosphorothioate (DEPT) were found in the urine of spray workers, who had been exposed to 8-hour time-weighted average airborne chlorpyrifos concentrations ranging from 0.012 to 0.145 mg/m^3. The authors claimed that most of these urinary alkylphosphates were derived from dermal absorption rather than from inhalation of chlorpyrifos (Sun89). In another exposure monitoring study, detectable concentrations of 3,5,6-TCP were measured in the urine of 6 termite control workers. Levels were lower than 0.10 mg TCP/g creatinine in the off-season period; however, they increased markedly in the beginning of the termite control season and reached peak levels ranging from 0.07 to 4.18 mg TCP/g creatinine in the busy spraying season. A significant negative correlation was found between urinary TCP levels and plasma cholinesterase activities (Jit89). Chlorpyrifos in serum and urinary metabolites DEP and DEPT were measured in 3 subjects following ingestion of a chlorpyrifos formulation. The subjects were admitted to hospital 2 to 5 hours

* at: http://www.ctb-wageningen.nl/geel.html.

after ingestion and initially treated with atropin and then during the first 5 days of hospitalisation with pralidoxime. The maximum levels of chlorpyrifos in serum and of urinary DEP and DEPT were measured on the day of admission. Chlorpyrifos was eliminated from the serum with a half-life of 1.1-3.3 h. The half-life of excretion of total urinary DEP + DEPT was initially 6 h, followed by a slower phase with a half-life of 80 h. The chlorpyrifos oxygen analogue chlorpyrifos oxon was not detected. There was no correlation between the maximum concentration of total urinary DEP + DEPT metabolites normalised to creatinine and the initial inhibition of blood cholinesterase activities (Dre93, Vas92).

Animal data

The inhalation absorption of chlorpyrifos vapours has been studied in 4 female F344 rats, following nose-only exposure to a concentration of 201 $\mu g/m^3$ for 6 hours. The average amount of 3,5,6-TCP and conjugates excreted in the urine during the first 48 hours after the beginning of exposure was 0.019 μg per 1 $\mu g/m^3$ of airborne chlorpyrifos exposure. From these data, the authors calculated that 72% of the inhaled vapours of chlorpyrifos was absorbed (Nol86).

Female Dublin ICR mice received a single dermal application of 1 mg/kg bw of ^{14}C-ring-labelled chlorpyrifos dissolved in acetone. The half-life of skin absorption (i.e., disappearance of radioactivity from the application site) of the chemical was 20.6 min. At 8 hours post-application, the percentage of skin penetration was 73.9%. At that time point, the radioactivity recovered in the urine and faeces was 13% and 26%, respectively, while that in carcass, blood, intestine, and liver amounted to 24%, 2.7%, 1.9%, and 1.8% of the applied dose, respectively (Sha81). To assess the dermal absorption of chlorpyrifos in rats, 0.2, 1.0, and 5.0% solutions of unlabelled compound in ethanol were applied under occlusion to the tail of Wistar rats for 4 hours. The amounts of chlorpyrifos found in tail tissues were 1.8, 2.4, and 2.5% of the applied dose, respectively (Tos94).

The oral absorption of chlorpyrifos was assessed in ICR mice that were given a single oral dose of 1 mg/kg bw of ^{14}C-ring-labelled chlorpyrifos by stomach needle. The half-life of absorption from the gastrointestinal tract into the body was 78 min. At 60 minutes post-application, the percentage of absorption was 47.2%, with 13.4 % of the radioactivity recovered in the urine (Ahd81). The percentage of absorption of the compound through ligatured stomach of ICR mice at 60 minutes post-application was only 11% (Ahd82).

In a metabolism and tissue distribution study, groups of male and female F344 rats were given single oral doses of 0.5 or 25 mg/kg bw of ^{14}C-labelled chlorpyrifos or 15 daily doses of 0.5 mg/kg bw unlabelled compound followed by one dose of 0.5 mg/kg bw of ^{14}C-labelled chlorpyrifos. During 72 hours, essentially all radioactivity had been recovered, mainly in the urine (84-92% of the administered dose), with 6-12% found in the faeces and less than 0.2% in the tissues (mainly perirenal fat, liver and ovaries) and the carcass. Repeated dosing induced a 6-7% increase in urinary excretion as compared with the single dose of 0.5 mg/kg bw. The major urinary metabolites were 3,5,6-TCP (12%), its glucuronide (80%), and, tentatively, a sulphate conjugate (Nol87). In another study, 12 male rats were given a single oral (gavage) dose of ^{14}C-ring-labelled chlorpyrifos of 5 mg/kg bw. Within 48 hours, 88% of the dose was excreted in the urine. At least 6 metabolites were detected, 3 of which accounted for 97% of the urinary radioactivity. These were identified as the glucuronide of 3,5,6-TCP (80%), a glucoside of TCP (4%), and freeTCP (12%) (Bak76). In a subsequent study with 4 female rats, given a single oral dose of ^{14}C-chlorpyrifos of 0.93 mg/kg bw, 79% of the dose was excreted in the urine and 15% in the faeces within 48 hours after the administration. Conjugated 3,5,6-TCP metabolites excreted in the urine would yield free 3,5,6-TCP on acid hydrolysis, indicating that the amount of total 3,5,6-TCP can be used to determine the amount of chlorpyrifos absorbed (Nol86).

Activation of chlorpyrifos by oxidative desulphuration to the active cholinesterase inhibitor, i.e., chlorpyrifos oxon, also occurs in animals but the compound is often not detected owing to its rapid rate of hydrolysis. The bulk of this bioactivation of chlorpyrifos occurs in the liver, and detoxification of chlorpyrifos and its oxon analogue in the liver, and plasma, producing hydrolysis products DEP, DEPT, and 3,5,6-TCP in free and conjugated form (Ric95, Rob99). Extensive work on chlorpyrifos biotransformation with mouse liver microsomes and perfused intact mouse liver indicates that the free oxon does not escape from the liver (Sul88, Sul91).

6 Effects and mechanism of action

Human data

Human data of acute and long-term health effects reported to be related to exposure to chlorpyrifos have been summarised in several reviews (Blo97, FAO00, Shu97).

Sensitisation

The prevalence of allergic contact dermatitis due to pesticide application was assessed by comparing 39 occupationally exposed subjects and 21 unexposed subjects by patch testing. Concerning chlorpyrifos, one out of 39 workers showed a positive reaction vs. none in the control group (Oma95).

Acute toxicity

In a case report of poisoning of a 42-year-old man with chlorpyrifos at an estimated dose of 300 mg/kg bw, inhibition of neuropathy target esterase (NTE) activity in lymphocytes was observed prior to the development of polyneuropathy. NTE is considered a molecular target in the nervous system for organophosphate-induced delayed polyneuropathy (OPIDN). Following ingestion of chlorpyrifos, cholinergic signs such as respiratory insufficiency, lachrymation, salivation, and fasciculations lasted for 17 days. During this period, extensive therapy with atropine and pyridine 2-aldoxime methane sulphonate (PAM) and artificial respiration was given. Thirty days after intoxication, the clinical and electrophysiological examination of the peripheral nervous system was normal, but plasma ChE, red blood cell acetylcholinesterase (AChE), and NTE activity were still inhibited by approximately 90%, 50%, and 60%, respectively. On day 43, weakness and paraesthesia in the legs were noted which became more severe over time. Clinical examination, electrophysiology, and a nerve biopsy revealed signs of a peripheral polyneuropathy with mild distal axonopathy, consistent with OPIDN (Lot84, Lot86). In another case report, a 38-year-old man drank an undefined quantity of a 25% chlorpyrifos solution. Cholinergic effects were stupor, respiratory distress, and complete inhibition of serum ChE activity. The man was successfully treated with extensive antidote therapy and artificial respiration and was discharged from the hospital one month after the incident. His serum ChE activity was still undetectable (She88). In a third case, a 21-year-old man who ingested an unknown quantity of chlorpyrifos showed cholinergic effects comprising pupillary constriction, excess secretions, tachycardia, and impaired consciousness. He was treated with atropine and PAM and received artificial respiration. Twenty-eight days after exposure, bilateral vocal cord paralysis developed which was considered to be indicative of OPIDN. The patient was asymptomatic within 4 weeks of the onset of symptoms (Sil94). In a fourth case, a pesticide applicator who had reportedly been exposed to chlorpyrifos in a closed environment for 6 months showed cholinergic signs,

including lachrymation, muscle twitching, and inhibition of red blood cell AChE. In addition, paraesthesias and numbness were reported. Neurological evaluation 6 weeks later revealed sensory loss of all modalities as well as mild distal weakness and decreased reflexes in the lower extremities, without fasciculations or tremors. Changes in neurological examination were consistent with polyneuropathy. Follow-up after 1 year revealed normalisation of the neurological examination and remission of all symptoms (Kap93).

In a human volunteer study, 6 male subjects were given a single oral dose of chlorpyrifos of 0.5 mg/kg bw. Four weeks later, 5 of the same men received a single dermal dose of 5 mg/kg bw of chlorpyrifos dissolved in dipropylene glycol methyl ether. No signs or symptoms of toxicity were reported in any of the men during the study. After oral administration, plasma ChE activity was maximally inhibited by 64-85% (mean 70%), 12 to 24 hours post-exposure, and red blood cell AChE by 11-52% (mean 27%), 4 days post-exposure. Plasma ChE activity had returned to more than 80% of the mean baseline level at day 30 after oral treatment. Men dermally exposed to 5 mg/kg bw exhibited a peak plasma ChE inhibition of 64-85% on post-exposure day 3 and returned to baseline values by day 40 of the study. Red blood cell AChE was not significantly inhibited after dermal dosing (Nol82, Nol84). In another human volunteer study, the effects of single oral doses of chlorpyrifos (purity: 99,8%) on red blood cell AChE activity was studied. Groups of 6 fasted men and women, aged 18-55 years, received single oral doses of 0, 0.5, 1, or 2 mg/kg bw in lactose powder. Blood samples for red blood cell AChE determination were collected pre-exposure and 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after treatment. Treatment had no effect on general health or on clinical chemical or haematological parameters measured 7 days after dosing. At 2 mg/kg bw, one woman exhibited a peak red blood cell AChE inhibition of 26% at 24 hours. The inhibition was 20% at 48 hours after treatment and no further samples were taken. The mean red blood cell AChE activity was not significantly different among treated groups and between treated groups and the 2 control groups at any time. The NOAEL for inhibition of red blood cell AChE following an acute oral dose is 1 mg/kg bw on the basis of significant inhibition in one of 12 subjects, and the NOAEL for clinical signs or symptoms was 2 mg/kg bw (Kis99).

Changes in the peripheral immune system were studied in 12 individuals, one to 4.5 years following exposure to chlorpyrifos after treatment of their homes or workplaces by licensed operators. In one subject, exposure took place by an accidental spill. No quantitative exposure measurements were reported. Control

groups comprised volunteer groups of 28 students and 29 home dwellers. A higher rate of atopy, antibiotic sensitivities, elevated CD26 cells (activated T cells), and autoimmunity was observed in the exposed group when compared to the control groups. The autoantibodies were directed toward smooth muscle, parietal cells, brush border, thyroid gland, myelin, and antinuclear antibody. The authors concluded that chlorpyrifos, as used in pesticide sprays, should be examined more closely as a probable immunotoxin (Thr93). In a subsequent study comprising 29 non-smoking individuals (10 males, 19 females), the results of the former study were confirmed. This group had chronic health complaints with symptoms such as flu-like illness, headache, loss of memory, dizziness, gastrointestinal disturbance, arthralgia, menstrual irregularities, fatigue, and heightened olfactory sensitivity to low concentrations of chemicals, and were diagnosed by their physicians as having multiple chemical hypersensitivity. The results of analyses of the peripheral blood markers peripheral lymphocyte phenotypes, autoantibodies, and mitogenesis to phytohaemagglutinin and concanavillin of this group were compared with the 2 volunteer unexposed and 1 exposed groups from the former study. Compared to the unexposed groups, there were changes in lymphocytes phenotypes, including an increase in CD26 expression and a decrease in percentage of CD5 phenotype, an increased frequency of autoantibodies, and a decreased mitogenesis in response to phytohaemagglutinin and concanavillin. These alterations in peripheral blood markers were unaffected by medication, age, sex, or season (Thr02).

Short-term toxicity

In a human volunteer study, 6 subjects were exposed to aerosols from a 63% concentrate of Dowco 179 in xylene by using a thermal fogging machine. Three subjects were exposed to an average air concentration of 2.7 mg/m^3, for 3 to 8 minutes, and 3 subjects to an average air level of 102 mg/m^3, for 1 to 4 minutes. Two subjects had a temporary decrease in plasma cholinesterase (ChE) activity (15 and 16% respectively) at 24 hours post-treatment. Activities, however, were already recovered at 96 hours post-exposure. No change in red blood cell acetylcholinesterase (AChE) activity was measured in any subject (Lud70).

In a dermal dose study, 5 men received single doses of chlorpyrifos ranging from 94 to 620 mg/kg bw (intermediate levels not given), for 12 hours. No skin irritation was recorded. Plasma ChE was significantly inhibited at the 2 highest doses, but no changes were observed in red blood cell AChE activity (Kil70).

In an oral study, male volunteers (n=4/dose) were given tablets of technical chlorpyrifos at doses of 0, 0.014, 0.03, or 0.1 mg/kg bw/day for 48, 27, 20, and 9 days respectively. After 9 days of treatment with 0.1 mg/kg bw/day, the mean plasma ChE activity of the 4 volunteers was decreased by 66% (range: -36 to -82% of baseline values) with respect to their pre-exposure values, while a mean increase of 20% was seen in the concurrent control group. The mean red blood cell AChE activity was increased by 5% (range: +2 to +9% of baseline values), while a mean increase of 14% was seen in the concurrent controls. One man developed blurred vision, runny nose, and a feeling of faintness, probably unrelated with exposure. No changes in clinical chemical and haematological analyses were found. Exposure of this group was discontinued because of the severe plasma ChE depressions, which returned to pre-exposure levels at 28 days after cessation of exposure. Men exposed to 0.03 mg/kg bw/day for 20 days showed a mean plasma ChE inhibition of 29% (range: -16 to -51% of baseline values), while the level in the concurrent control group was increased by 7%. A steady state inhibition of plasma ChE was reached after about 16 days of exposure. Plasma ChE activities had been returned to pre-exposure values at 28 days after cessation of treatment. The mean red blood cell AChE activity was increased by 1.5% (range: -5 to +6% of baseline values), compared with an increase of 3% in the concurrent control group. Even at 0.014 mg/kg bw/day, the mean plasma ChE activity of the men was still decreased by 6.5% (mean: -20 to +21% of baseline values), accompanied by a 6.5% increase in the concurrent controls. However, the difference was not statistically significant. Mean red blood cell AChE activity was increased by 2.5 % (range: -10 to +10% of baseline values) (Cou72, Gri76). According to the committee, the NOAEL for inhibition of plasma ChE after 28 days of oral exposure to chlorpyrifos is 0.014 mg/kg bw/day. The NOAEL for red blood cell AChE is greater than 0.014 mg/kg bw/day.

Several field studies were carried out to assess the health implications following application of chlorpyrifos. Five out of 7 spray workers, applying a 0.5% emulsion or suspension of water-wettable powder for control of pest mosquitoes, showed decreases in plasma ChE of 50-70% of baseline values within 2 weeks after beginning the work. No inhibition of red blood cell AChE was observed at any time and there were no signs or symptoms of illness (Eli69). In other studies, plasma ChE or red blood cell AChE were measured in pest control operators, applying a range of organophosphorus pesticides, among which chlorpyrifos. The principle mechanism of toxicity for all these compounds

is inhibition of cholinesterase activity, so that the effect of a single compound, e.g., chlorpyrifos could not be assessed (Hay80, Yea93).

Long-term toxicity

In a cohort study, the prevalence of selected illnesses and symptoms was compared between 175 employees potentially exposed to chlorpyrifos and 335 matched controls with no history of exposure to organophosphorus pesticides. Chlorpyrifos workers had held jobs between 1 January 1977 and 31 July 1985. Control subjects were matched on age, race, sex, starting date of employment, and pay status. Morbidity data were abstracted from company medical records. Subjects were subdivided into 3 exposure groups (high, moderate, low) on the basis of job title and air monitoring data. The time-weighted average (times not given) airborne exposures to chlorpyrifos, measured by personal air sampling during manufacture and formulation over the period 1979 to 1983, were between <0.03 and 0.12 mg/m^3. However, peak exposures as high as 1.1 mg/m^3 were measured. No information was given on air exposure levels in the 3 groups. Plasma ChE activity was measured in workers at monthly intervals as an indicator of exposure to chlorpyrifos. In comparison with pre-employment levels, mean plasma ChE activities of workers subdivided into the 3 exposure categories were inhibited by 32% (high), 32% (moderate), and 19% (low). No significant differences in illness or prevalence of symptoms between the exposed and control groups, or among the 3 exposure subgroups were observed. Potentially exposed employees did report symptoms of dizziness, malaise, and fatigue relatively more often than subjects from the control group. Further analysis by estimated exposure level, process area, or time did not support a relation with exposure. No cases of peripheral neuropathy were seen among the exposed workers (Bre89). In a follow-up to this study, the data were updated to include the period 1987-1994, and additional medical disorders (e.g., disorders of the central nervous system and of the eye and adnexa and diseases of the ear and the mastoid process) were considered. The updated study comprised 496 potentially exposed employees and 911 controls matched for age, race, sex, pay, year of hire, and smoking and drinking habits. Workers were subdivided into 4 categories of chlorpyrifos exposure: ‘high’ (average air levels >0.2 mg/m^3, or high dermal exposure), ‘moderate’ (average air levels between 0.03 and 0.2 mg/m^3, or moderate dermal exposure), ‘low’ (average air levels between 0.01 and 0.03 mg/m^3, or low dermal exposure) or ‘negligible’ (average air levels<0.01 mg/m^3, or negligible dermal exposure). Plasma ChE activity was measured in

workers at monthly intervals as an indicator of exposure to chlorpyrifos. In comparison with pre-employment levels, mean plasma ChE activities of workers subdivided into the 4 exposure categories were inhibited by approximately 45% ('high'; n=1), 20% ('moderate'; n=324), and 5% ('low'; n=110) while an increase of 20% in plasma ChE activity was seen in the 'negligible' exposure group (n=29). The results of morbidity showed that the prevalence of peripheral neuropathy was not significantly increased among the group of employees potentially exposed to chlorpyrifos. Significantly increased prevalence odds ratios were identified for 5 diagnostic categories: diseases of ear and mastoid process; acute respiratory infections; other diseases of the respiratory system; general symptoms, signs, and ill defined conditions; and symptoms, signs, and ill defined conditions involving the digestive system. Analyses by exposure classification or mean plasma ChE activity did not show a dose response (Bur98).

In a recent unpublished prospective cohort study, neurobehavioural examinations and neurological examinations that included evaluation of central and peripheral nervous system function were conducted on 53 workers of the same chemical company, engaged in the manufacture of chlorpyrifos. A group of 60 workers, not occupationally exposed to chlorpyrifos or potential neurotoxic agents, was chosen as a referent population. Data on years of employment of workers were not given. Subjects were examined on 2 occasions at 1-year intervals (autumn 1999 and autumn 2000). Data collected were a general medical examination, a neurological examination, an electrodiagnostic evaluation, a psychological interview, neurobehavioural testing, and measurement of red blood cell AChE activity. In addition, monthly measurements of plasma ChE activity were conducted. Industrial hygiene measurements of airborne chlorpyrifos concentrations were conducted to evaluate potential worker exposure during the study period, and overall chlorpyrifos absorption into the worker's body was assessed by the measurement of timed overnight urinary 3,5,6-TCP excretion on 4 occasions. Urine was collected at the time of the 1999 and 2000 clinical examination, in autumn 2000 during the chlorpyrifos maintenance shutdown, and in autumn 2000 during normal plant operations. The mean cumulative airborne chlorpyrifos exposure during the study period (about 225 days) was 6.13 mg/m^3•day among chlorpyrifos workers, corresponding with an average daily exposure of approximately 0.027 mg/m^3, compared with 0.0 mg/m^3•day in the referent group. The mean cumulative historic exposure was 64.2 mg/m^3•day among chlorpyrifos workers, compared with 0.7 mg/m^3•day among the referent group. The 1-year weighted average urinary 3,5,6-TCP

concentration of chlorpyrifos workers (n=51) was 192 μg/g creatinine (range: 9 to 1536 μg/g creatinine), compared with 6.2 μg/g creatinine (range: 1.5 to 12 μg/g creatinine) among the referent group (n=60). The cumulative airborne chlorpyrifos exposure during the study was significantly correlated with urinary 3,5,6-TCP concentration, suggesting that skin absorption was minimal. The average plasma ChE level was statistically significantly decreased by 13% among chlorpyrifos workers, compared with the referent group. There were statistically significant inverse correlations between airborne chlorpyrifos or urinary 3,5,6-TCP concentrations and plasma ChE activity. The authors suggested that no effect on plasma ChE occurred at ambient exposures below 0.11 mg/m^3 or urinary 3,5,6-TCP concentrations below 12 μg/g creatinine. No statistically significant difference was found in the average red blood cell AChE activity among chlorpyrifos and control workers, and no correlation was found between urinary 3,5,6-TCP concentration and red blood cell AChE activity. According to the committee, this implies that at 1-year weighted average 3,5,6-TCP excretions, ranging from 9 to 1536 μg/g creatinine, corresponding to absorbed chlorpyrifos doses of approximately 0.03 to 3.7 mg/day (or 0.0003 to 0.043 mg/kg bw/day), no significant inhibition of red blood cell AChE activity occurs. With regard to the neurobehavioural examinations, no dose-related subclinical or clinically evident adverse neurobehavioural effects were found. No statistically significant changes in central and peripheral nervous system function were found in chlorpyrifos workers compared to the referent group (Alb02).

From these data the committee concludes that the NOEL for inhibition of plasma ChE is between 0.01 and 0.11 mg/m^3 chlorpyrifos. However, from data generated in the above short-term human volunteer study and the recent prospective cohort study, the committee considers the NOAEL for inhibition of red blood cell AChE to be substantially higher than the NOEL for inhibition of plasma ChE. In addition, the committee does not exclude that the observed plasma ChE inhibitions may be partly due to dermal absorption rather than to inhalation of chlorpyrifos, as demonstrated in other exposure monitoring studies (Jit89, Sun89).

Developmental toxicity

Four cases of birth defects allegedly associated with exposure to chlorpyrifos were reported. The children were found to have defects, including the brain, eyes, ears, palate, teeth, heart, feet, nipples, and genitalia. Brain defects were

present in the ventricles, corpus callosum, choroid plexus, and septum pellucidum, and genital defects included the testes (not descended), microphallus and fused labia. All children had growth retardation and 3 had hypotonia and profound mental retardation. The mothers of the affected children had reportedly been exposed to chlorpyrifos, one of them at the workplace and the others at home during pregnancy. The exposure patterns were poorly characterised and it was not indicated whether it was severe or sustained during organogenesis. Medical records of the cases indicated that the same effects in some of the children were consistent with a diagnosis of an autosomal recessive birth defect syndrome of the brain and eye (Gib96, She96).

Animal data

Irritation and sensitisation

Chlorpyrifos is slightly irritating to the eyes and the skin of rabbits, using standard tests (FAO00). In a 4-day dermal study in rabbits, slight erythema in 2/4 females was observed at 1 and 10 mg/kg bw/day (Cal88, Cal89). The compound was not a skin sensitiser in the standard Magnusson-Kligman or Buehler tests (FAO00).

Acute toxicity

Results of acute lethal toxicity tests are summarised in Table 1.

Table 1 Summary of acute toxicity studies for chlorpyrifos in mammals.

exposure route	conditions	species (strain)	sex	LC_{50} or LD_{50}	reference
inhalation[a]	vapour; 4 h; nose only	rat (Sprague-Dawley)	male, female	>36 mg/m^3	Bla94
	undiluted; 4 h; nose only; powder	rat (Sprague-Dawley)	male, female	>230 mg/m^3	And95
	aerosol; undiluted; 4 h; whole body	rat (Wistar)	male, female	>1020 mg/m^3	Ken87
	aerosol; undiluted; 4 h; whole body	rat (Wistar)	male, female	560 mg/m^3	Fre96
dermal	in xylene	rat (Sherman)	male	202 mg/kg bw	Gai69
	in arachis oil	rat (Sprague-Dawley)	male, female	>2000 mg/kg bw	Dre94b
	in polyethylene glycol	rat (Sprague-Dawley)	male.female	>2000 mg/kg bw	Lac85
	undiluted	rat (Sprague-Dawley)	male.female	>2000 mg/kg bw	Jac94

	undiluted	rat	male, female	>2000 mg/kg bw	Nis84a
	undiluted	rat (F344)	male, female	>2000 mg/kg bw	Jef86
	in saline	rat (Sprague-Dawley)	male, female	>5000 mg/kg bw	Buc81b
	in water	rabbit (Himalayan)	male, female	1233 mg/kg bw	Fre95a
	undiluted	rabbit (New Zealand White)	male, female	1580-1801 mg/kg bw	Hen80
oral	in peanut oil	rat (Sherman)	male	155 mg/kg bw	Gai69
	in peanut oil	rat (Sherman)	female	82 mg/kg bw	Gai69
	in corn oil	rat (Sherman)	male	118, 135 mg/kg bw	McC74
	in corn oil	rat (Sherman)	female	155 mg/kg bw	McC74
	in corn oil	rat (Dow-Wistar)	male	163, 245 mg/kg bw	McC74
	in corn oil	rat (Dow-Wistar)	female	135 mg/kg bw	McC74
	in soya bean oil	rat (Sprague-Dawley)	female	169 mg/kg bw	Ber78
	in arachis oil	rat (Sprague-Dawley)	male	276 mg/kg bw	Dre94a
	in arachis oil	rat (Sprague-Dawley)	female	350 mg/kg bw	Dre94a
	in maize oil	rat (Sprague-Dawley)	male	264 mg/kg bw	Wil94
	in maize oil	rat (Sprague-Dawley)	female	141 mg/kg bw	Wil94
	in maize oil	rat (Sprague-Dawley)	male	475 mg/kg bw	Buc81a
	in maize oil	rat (Sprague-Dawley)	female	337 mg/kg bw	Buc81a
	in corn oil	rat (Sprague-Dawley)	male	221 mg/kg bw	Nis84b
	in corn oil	rat (Sprague-Dawley)	female	144 mg/kg bw	Nis84b
	in corn oil	rat	male	163 mg/kg bw	Tay63
	in corn oil	rat	female	135 mg/kg bw	Tay63
	in vegetable oil	rat (Wistar)	male, female	134 mg/kg bw	Fre95b
		rat (Sprague-Dawley)	male	205-270 mg/kg bw	Hen80
		rat (Sprague-Dawley)	female	96-174 mg/kg bw	Hen80
	in aqueous gum tragacanth	mouse (Swiss-Webster)	male	102 mg/kg bw	Cou71
	in soya bean oil	mouse (NAMRU)	female	152 mg/kg bw	Ber78
	in vegetable oil	mouse (Swiss albino)	male, female	109 mg/kg bw	Tay63
	in corn oil	guinea pig	male	504 mg/kg bw	McC74

[a] The inhalation studies were carried out at the highest attainable vapour or aerosol concentrations.

Single oral doses of chlorpyrifos (in corn oil) of 500, 1000, or 2000 mg/kg bw caused mortality in 0/2, 0/2, and 2/2 New Zealand White rabbits (sex: not reported), respectively (McC74).

From the results in Table 1, the committee concludes that there is considerable variation in the susceptibility of different species. Mice are the most sensitive and rabbits the less sensitive species. Females are more sensitive than males.

In adult rats receiving single oral doses of 0.5, 1, 5, 10, or 100 mg/kg bw chlorpyrifos (purity: 89-99%), NOAELs of 10 and 0.5 mg/kg bw were found for inhibition of brain AChE and plasma ChE, respectively (Men98). When rats were given chlorpyrifos by subcutaneous injection at a dose of 280 mg/kg bw, marked inhibition of AChE activity (>90%) in the cerebral cortex and corpus striatum was measured, which did not recover within 14 days after dosing. The changes in AChE activity were accompanied by reductions in muscarinic receptor binding sites in the cortex and striatum (Cha93).

In an acute neurotoxicity study, rats given single oral doses of 0, 10, 50, or 100 mg/kg bw chlorpyrifos (purity: 98.2%) exhibited decreased body weight, motor activity, and clinical signs of intoxication at 50 and 100 mg/kg bw. The NOAEL for systemic effects was 10 mg/kg bw (Wil92).

In a neurobehavioural study, as determined by observational tests of function and motor activity, adult male Long-Evans rats (n=10/group) that received single oral (gavage) doses of 0, 20, 50, and 100 mg/kg bw chlorpyrifos (purity: 99.5%) showed autonomic activity, e.g., salivation, lachrymation, miosis, and mild tremors, altered neuromuscular function (gait changes), and decreased motor and sensorimotor activity. The most sensitive effect was a change in motor activity, which was depressed at 20 mg/kg bw. The rats recovered from the effects at 72 hours after dosing (Mos95). In a subsequent neurobehavioural study by the same author, adult male Long-Evans rats (n=20/group) were given single oral (gavage) doses of 0, 10, 30, 60, or 100 mg/kg bw of chlorpyrifos (purity: 99.5%). Clinical and neurobehavioural signs, i.e., increased autonomic and convulsive activity and decreased motor, neuromuscular, and sensorimotor activity were observed at doses of 30 mg/kg bw and above, at 3.5 hours after dosing. At these doses, brain AChE and whole blood ChE activities were inhibited by approximately more than 60% and 80%, respectively. At 24 hours, there was partial recovery from behavioural effects, i.e., considerably fewer behavioural endpoints were affected and most of them were seen in the 2 higher dose groups only, with little or no corresponding recovery of ChE activity. Body temperature, gait, and motor activity appeared to be the most sensitive measures. At 10 mg/kg bw, the lowest dose tested, brain AChE activities were inhibited by approximately 30 and 40% 3.5 and 24 hours after dosing, respectively, and whole blood ChE activities by about 80 and 35%, respectively, but no behavioural effects were observed.

Relating incidence of behavioural effects to the extent of ChE inhibition (instead of by dose) in individual rats suggested that single dosing of chlorpyrifos did not induce clinical signs when brain AChE and whole blood ChE activities were inhibited by less than 60 and 80%, respectively (Nos97). Sprague-Dawley rats (n=5/sacrifice group; sacrifices 2, 4, 6, and 12 weeks post-treatment) that received a single subcutaneous dose of 279 mg/kg bw (estimated to be the maximum tolerated dose; see Pop91) of chlorpyrifos (purity: 98%) showed inhibition of AChE activity in cerebral cortex and corpus striatum by approximately 95% and 60% at weeks 2 and 6 after dosing, respectively. AChE activities had returned to normal values at 12 weeks after dosing. Tests of locomotor activity showed hypoactivity in treated rats for 2-3 days after treatment (Pop92). Long-Evans rats, trained to perform an appetitive test of memory and motor function, received a single subcutaneous injection with 0, 60, 125, or 250 mg/kg bw chlorpyrifos (purity: not given). Seven days post-treatment, statistically significant, dose-related reductions in AChE activities were observed in cerebral cortex, hippocampus, corpus striatum, and hypothalamus. Apart from the activity in the hypothalamus at 60 mg/kg bw, these AChE activities were still statistically significantly lower when compared to controls 21 days post-treatment although partial recovery had occurred. AChE activity in whole blood was inhibited 4 days after treatment. Activity slowly returned to control levels in the low-dose animals by day 53 and in the mid-dose animals by day 74. In the high-dose group, there was still a small, but statistically significant decrease 53 days post-treatment. At the 2 highest dose levels, effects on working memory and motor function appeared within 2 days after dosing, but returned to normal within 3 weeks (Bus93).

The potential of chlorpyrifos to inhibit neuropathy target esterase (NTE) was studied in Fischer 344 female rats that were given single oral (gavage) doses of 0, 1, 5, 10, 50, or 100 mg/kg bw of chlorpyrifos (purity: 98.1%). NTE was not inhibited at any dose. The NOAEL was 10 mg/kg bw for inhibition of brain AChE, and 1 mg/kg bw for inhibition of red blood cell AChE and plasma ChE (Dit97).

Chlorpyrifos was tested for acute delayed neurotoxicity in several studies with hens. In all studies, chlorpyrifos was given together with atropine and/or PAM to protect the animals from the acute effects of the compound. In hens treated with a single dose of 110 mg/kg bw of chlorpyrifos (purity: 96.8%), no clinical signs of delayed neurotoxicity and no microscopic lesions of nerve tissues characteristic of OPIDN were found (Rob87). Hens that were given oral doses of chlorpyrifos (purity: 99%) ranging from 60 to 150 mg/kg bw exhibited

inhibition of 59-97% of brain NTE and of 75-100% of brain AChE activity, 4-6 days after dosing. Delayed polyneuropathy was already observed at 60-90 mg/kg bw, which corresponds to 4-6 times the LD_{50} (Cap91). In another study, hens receiving oral doses of chlorpyrifos (purity: 100%) at 0, 75, 150, or 300 mg/kg bw exhibited brain AChE depressions of 0, 58, 75, and 86% and brain NTE depressions of 0, 21, 40, and 77% respectively, 4 days after treatment (Ric93).

The effect of chlorpyrifos on the production of hepatic and brain lipid peroxidation was studied in female Sprague-Dawley rats, following 2 equal oral doses of 41 mg/kg bw chlorpyrifos (purity: 97%) in corn oil at 21-hour intervals. A 4 to 5-fold increase in lipid peroxidation was measured in both hepatic and brain homogenates, probably resulting from enhanced production of reactive oxygen species (Bag95).

The effect of age on the toxicity of chlorpyrifos has been reported in several studies in the peer-reviewed literature. Rats given subcutaneous injections of 2 mg/kg bw chlorpyrifos on post-natal day 1, showed significant inhibition of DNA and protein synthesis (10 and 30%, respectively) in all brain regions within 4 hours of treatment. Comparable inhibition of DNA synthesis was seen at 8 days of age, at 11 mg/kg bw/day, but here regional selectivity was observed. Effects on protein synthesis was distinct from that on DNA synthesis, as it had diminished to <10% by 8 days of age and did not show regional selectivity. The authors argued that this effect was not secondary to systemic intoxication (Whi95). The maximum tolerated dose of chlorpyrifos in Sprague-Dawley rats injected subcutaneously was 45 mg/kg bw in 7-day-old pups and 279 mg/kg bw in adults (90-day old). Neonatal brain AChE inhibition was 80% on day 1 and 45% on day 7 after treatment, whereas 90% inhibition was seen in adult rats, with little or no recovery on day 7 (Pop91). The committee concludes that the young rat will respond more to the anticholinesterase effects of chlorpyrifos than adult animals.

Subacute and subchronic toxicity

Exposure of Fischer 344 rats (numbers not given) that received chlorpyrifos (purity: 95%) by nose-only inhalation, 6 hours/day, for 5 days, at a concentration of 0.34 mg/m^3, resulted in a significant decrease in plasma ChE activity in females, but no effects were seen in red blood cell or brain AChE activities (New88a). Nose-only exposure of female Fischer rats (numbers not given) to 0.172 mg/m^3 of chlorpyrifos for 2 weeks (6 hours/day, 5 days/week) did not result in mortality, cholinergic signs of toxicity, changes in clinical chemistry and

haematological parameters, or inhibition of plasma ChE, red blood cell AChE, or brain AChE (Lan86). In a 2-week whole-body inhalation study, Fischer 344 rats (numbers not given) were exposed to 0, 0.014, and 0.072 mg/m^3 chlorpyrifos vapour (6 hours/day, 5 days/week). There were no treatment-related mortality, clinical signs of toxicity, or changes in body weight, and no treatment-related changes in organ weights or microscopic abnormalities. No effects were observed on brain and red blood cell AChE or plasma ChE activities (Str87). In another 2-week whole-body inhalation study, Wistar rats (numbers not given), exposed to chlorpyrifos at concentrations 0, 10, 94, and 388 mg/m^3 (6 hours/day, 5 days/week), showed high mortality at the high dose. At 388 and 94 mg/m^3, cholinergic signs of toxicity, decreased body weights and increased adrenal weights were seen. There was no NOAEL for inhibition of cholinesterases in this study. At the lowest concentration, brain AChE was inhibited by 50% (males and females), red blood cell AChE by 78% in males and 60% in females, and plasma ChE by 78% in males and 91% in females (Ken88).

Two identical 13-week inhalation studies were conducted in Fischer 344 rats. In the first study, groups of 10 rats/sex/group were exposed nose-only to concentrations of 0, 0.072, 0.143, or 0.287 mg/m^3 (the highest attainable) of chlorpyrifos vapour (purity: 100%), 6 hours/day, 5 days/week. There were no treatment-related signs of toxicity during the course of the study. No abnormalities were observed in urine analysis, haematology, clinical chemistry, organ weights, or upon gross and microscopic examination. No differences in plasma ChE, in red blood cell, and brain AChE activities were noted between any of the exposed groups and the control group (Cor89). In the second study, groups of 15 rats/sex/group received nose-only exposure to 0, 0.07, 0.14, or 0.28 mg/m^3 of chlorpyrifos (purity: 95%), for 13 weeks. There were no treatment-related mortality or ophthalmic, haematological, or clinical chemical changes. At the high dose, plasma ChE activity was inhibited by 23% in males. Brain AChE and red blood cell AChE were unaffected (New88b). The committee concludes that the NOAEL for inhibition of brain AChE or red blood cell AChE was greater than the highest attainable concentration of chlorpyrifos vapour, i.e., >0.287 mg/m^3.

Fischer 344 rats (numbers not given) were treated dermally with chlorpyrifos (purity: 100%) at doses of 0, 1, 10, 100, or 500 mg/kg bw/day in corn oil, for 4 days. No clinical signs of intoxication were observed. At 10 mg/kg bw, red blood cell AChE activity was inhibited by 16% and plasma ChE by 45%. The same authors conducted a 3-week dermal study with chlopyrifos in rats at 0, 0.1, 0.5, or 5 mg/kg bw in corn oil, for a total of 15 applications. No treatment-related

cholinergic signs were observed at any dose, and plasma ChE, red blood cell AChE, and brain AChE activities were not affected. Gross and microscopic examination did not reveal treatment-related changes. The NOAEL was 5 mg/kg bw, based on inhibition of red blood cell AChE (Cal88, Cal89).

Groups of 20 Sprague-Dawley rats of each sex were given chlorpyrifos (purity: not given) in the diet at levels equivalent to 0, 0.03, 0.15, or 0.75 mg/kg bw, for 6 months. No treatment-related changes in body weight gain, food consumption, or haematological or clinical chemical parameters were observed. At 0.75 mg/kg bw/day, red blood cell AChE and plasma ChE activities were inhibited by 50% and 65%, respectively. Brain AChE activity was not inhibited at any dose level (Cou71). In a 13-week oral study, Sprague-Dawley rats (n=20/sex/group) were fed levels of chlorpyrifos (purity: 95%) equivalent to 0, 0.025, 0.5, or 10 mg/kg bw/day. No deaths or clinical signs were observed at any dose and no treatment-related abnormalities were observed in clinical chemical parameters and upon ophthalmoscopic examination. However, at 10 mg/kg bw/day, reduced body weights, haemoglobin levels, and erythrocyte counts were measured. Brain and red blood cell AChE activities were not measured in this study. The NOAEL for systemic effects was 0.5 mg/kg bw/day (Cro88). In another 13-week feeding study, Fischer 344 rats were given chlorpyrifos (purity: 96%) at doses equivalent to 0, 0.1, 1, 5, or 15 mg/kg bw/day. At 15 mg/kg bw/day, treatment-related effects included decreased body weight gain, decreased erythrocyte count, increased platelet counts, reduced serum protein, albumin and globulin levels, increased organ weights, and increased adrenal gland vacuolation. Plasma ChE and red blood cell AChE were inhibited at 1 mg/kg
bw and above, and brain AChE in both sexes at 5 and 15 mg/kg bw/day. The NOAEL was 0.1 mg/kg bw/day, based on inhibition of red blood cell AChE (Sza88).

In a 4-week neurotoxicity study, chlorpyrifos (purity: 98%) was fed to Long-Evans rats (n=10/sex/group) at doses equivalent to 0, 1, 3, or 10 mg/kg bw/day. Cholinergic signs of intoxication were observed at 10 mg/kg bw/day (salivation and tremors) and at 3 mg/kg bw/day (miosis). Brain AChE, red blood cell AChE, and plasma ChE activity were inhibited by more than 60% at 3 and 10 mg/kg bw/day. At the lowest dose, brain AChE was not significantly reduced (8%), but red blood cell AChE and plasma ChE were still inhibited by 56% and 68%, respectively. Neuropathy target esterase (NTE) was decreased by 6% in the high-dose group, but the committee did not consider this as toxicologically significant. Cognitive function, as measured by short-term memory and attention/encoding

deficits, was not impaired at any of the dose levels (Mat96). In a 13-week neurotoxicity study, Fischer 344 rats (n=10/sex/group) were fed chlorpyrifos (purity: 98%) at doses equivalent of 0, 0.1, 1, 5, or 15 mg/kg bw/day. A functional observational battery of tests (FOB), ophthalmic examination, and gross and microscopic examination of nerve tissues were conducted. FOB, conducted before dosing and at weeks 4, 8, and 13, consisted of observations of cholinergic signs, measurement of grip performance, and landing foot splay. A slight reduction in motor activity was observed at the top dose during week 4 only, and increased urine incontinence was observed in several females in the 2 highest dose groups at weeks 4, 8, and 13. According to the authors, these transient effects were not treatment-related. Neuropathological examination did not reveal any differences, which might be attributed to treatment. Cholinesterase activities were not measured. The NOAEL for neurotoxic effects was >15 mg/kg bw/day (Sha93).

CD-1 mice (n=40/sex/group) were given chlorpyrifos (purity: 96%) in the diet at levels equivalent to 0, 2.7 (males), or 3.4 (females) mg/kg bw/day, for 4 weeks. No mortality, clinical signs, or abnormalities in gross and microscopic examination were observed. Male body weight gain was reduced by 25% at the end of the study. At termination, red blood cell AChE was depressed by 53% and plasma ChE by 91%, but brain AChE remained unchanged (Dav85). Groups of CD-1 mice (n=12/sex/group) were fed chlorpyrifos (purity: 94%) at levels equivalent to approximately 1, 10, 40, 90, or 200 mg/kg bw, for 13 weeks. Dose-related increases in mortality rate and in the frequency of ocular opacities were observed at the 2 highest dose levels. At termination, plasma ChE activity was significantly depressed at all doses in both males and females. Red blood cell AChE activity was significantly inhibited in females at 10 mg/kg bw/day and above, but remained unaffected in males at any dose level. Brain AChE activity was depressed at 10 mg/kg bw/day and above in females and at 40 mg/kg bw in males. The absolute and relative organ weights remained unaffected, except for an increase in the relative liver weight. Microscopic examination showed abnormalities in the adrenal glands (lipogenic pigmentation) at 40 mg/kg bw/day and above, and in the eyes (keratitis) at the top dose. The NOAEL was 1 mg/kg bw/day, based on inhibition of brain and red blood cell AChE (Cro87).

In a recent 6-week study, beagle dogs (n=4/sex/group) received chlorpyrifos (purity: 97.6%) via the diet at doses equivalent to 0.0, 0.5, 1.0, or 2.0 mg/kg bw/day for males, and 0.0, 0.5, 1.1, or 1.9 mg/kg bw/day for females. No mortality or clinical signs of intoxication were observed in any of the groups. There were no statistically significant differences in mean body weight and body

weight gain, mean food consumption, macroscopic examination of organs, or microscopic examination of the adrenal glands between the treatment and control groups. At 18 hours after the beginning of feeding, combined male and female mean red blood cell AChE activities were statistically significantly inhibited by 11 and 10% in the mid- and high-dose animals, respectively. Progressive inhibition of red blood cell AChE activity was apparent after 1 week of exposure, and a steady state inhibition was reached after 3 weeks of exposure. Terminal mean red blood cell AChE activities, measured immediately after necropsy, were depressed by 53, 71, and 85% (males), and by 38, 64, and 80% (females) for low-, mid-, and high-dose animals, respectively, with respect to mean baseline and control values. Brain AChE activity, measured immediately after necropsy, was 97, 90, and 92% (males), and 104, 101, and 96% (females) of controls for the low-, mid-, and high-dose groups. The change in the mid-dose males was statistically significant. However, the inhibition was not dose related, and according to the committee, the mid-dose finding was due to normal variability rather than a treatment-related effect. When male and female data were combined, brain AChE activity in the low-, mid-, and high-dose groups were inhibited by 0, 4, and 6%, respectively. Because the AChE was inhibited in both the high-dose males and females, the committee considered the change as a treatment-related effect. No statistically significant inhibitions were found in male or female left atrium, diaphragm, quadriceps, or nodose ganglion AChE activities in any of the treatment groups (Mar01, Ste01). According to the committee, the NOAEL for brain and peripheral tissue AChE inhibition was 1.0 mg/kg bw/day. No NOAEL could be set for red blood cell AChE (LOAEL: 0.5 mg/kg bw/day). The NOAEL for red blood cell AChE after 1-day exposure was set at 0.5 mg/kg bw/day.

In another study, beagle dogs (n=4/sex/group) were given chlorpyrifos (purity: 96%) at doses of 0, 0.01, 0.22, or 5 mg/kg bw/day by capsule, for 13 weeks. No clinical signs of toxicity, haematological, clinical chemical, ophthalmoscopic, gross, or microscopic abnormalities were observed. Dose-related inhibitions were observed in plasma ChE activity at 0.01 mg/kg bw/day and above, in red blood cell AChE at 0.22 mg/kg bw/day and above, and in brain AChE (by 46%) at 5 mg/kg bw/day. The NOAELs for red blood cell AChE and brain AChE were 0.01 and 0.22 mg/kg bw, respectively (Har89).

Beagle dogs (n=3/sex/group) received diets containing chlorpyrifos (purity: 97%) at levels equivalent to 0, 0.01, 0.03, 0.1, 1.0, or 3.0 mg/kg bw/day for 1 year. The same doses were given to 4 beagles/sex/group for 2 years. No treatment-related mortality, signs of toxicity, or abnormalities in food

consumption, body weights, haematological or clinical chemical parameters were observed. No differences in absolute and relative organ weights were found between controls and treated dogs, except for an increase in relative liver weight at the top dose. Gross and microscopic examination of the tissues revealed no treatment-related alterations. Progressive inhibition of red blood cell AChE activity was apparent after 1 week of exposure, and a steady state inhibition was reached after 30 days of treatment in both studies. Statistically significant red blood cell AChE depressions were found for the groups of dogs receiving doses of 1.0 and 3.0 mg/kg bw/day, with values ranging from 61 to 75% in males and 60 to 70% in females, respectively. Brain AChE activity was inhibited at 3 mg/kg bw/day only (21% in males and 19% in females), but the difference with the control group was not statistically significant. Plasma ChE activity was significantly decreased at 0.1 mg/kg bw/day and above in both males (49-75%) and females (35-64%) at all assay times. The NOAELs for inhibition of red blood cell and brain AChE were 0.1 and 1.0 mg/kg bw, respectively (Mat01, McC74).

Groups of rhesus monkeys (n=1-2/sex/group) received chlorpyrifos (purity: not given) at doses of 0, 0.08, 0.4, or 2 mg/kg bw/day by stomach tube, for 6 months. No treatment-related changes in body weight gain, food consumption, haematological and clinical chemical parameters, or gross and microscopic examination were observed. At week 24, plasma ChE and red blood cell AChE activities were significantly inhibited at 0.4 mg/kg bw/day (by 61% and 27%, respectively) and at 2 mg/kg bw/day (by 55% and 38%, respectively). At termination, brain AChE activity was not significantly inhibited at any dose level (Cou71, FAO00).

Male Long-Evans rats (n=7-8/group) were given chlorpyrifos (purity: not given) by subcutaneous injection at doses of 0, 15, 30, and 60 mg/kg bw in peanut oil, for 20 weeks. Injections were given at weekly intervals during weeks 1-5, at 14-day intervals during weeks 6-10, and then weekly dosing was reinstated for weeks 11 to 20. Whole blood ChE activity was depressed by 60% (at 15 mg/kg bw) to 90% (at 60 mg/kg bw) after 5 weeks. At 60 mg/kg bw, tremors were observed as well as working memory impairment and slowing on motor activity. Reducing the chlorpyrifos injection frequency relieved the inhibition of whole blood ChE activity to 50%-75% of control and cholinergic signs, and behaviour returned to normal levels. Reinstatement of weekly injections for 10 weeks inhibited whole blood ChE by 75% to 90%. Tremor was not observed, but motor slowing and working memory impairment persisted throughout the dosing period in all treated groups. The authors conclude that

repeated chlorpyrifos injections lead to persistent impairment of cognitive and motor function, while tolerance to the muscarinic effects of chlorpyrifos was observed (Bus94).

Adult white Leghorn hens (n=15) were given chlorpyrifos (purity: 100%) orally for 20 days at a dose of 10 mg/kg bw/day, followed by a 4-week observation period. Body weights were decreased by 25% and some birds showed clinical signs of toxicity during week 1 of treatment. During days 4-20, brain AChE activity was depressed by 58-70%, but returned to normal values after the 4-week observation period. NTE activity in brain and lymphocytes throughout the study was 82-99% and 85-128% of that of controls, respectively. The 18% inhibition of brain NTE on days 10 and 20 was statistically significant. No behaviour signs of OPIDN were observed (Ric93).

The results of subacute and subchronic toxicity studies in rats, mice, dogs, monkeys, and chickens are summarised in Table 2.

Table 2 Summary of subacute and subchronic toxicity studies for chlorpyrifos.

exposure route	species (strain; number; sex)	dose levels	exposure duration	critical effect[a]	NOAEL	reference
inhalation	rat (F344; females; n= ?)	0.34 mg/m^3 (nose only)	5 days	BAChE; RAChE	>0.34 mg/m^3	New88a
	rat (F344; female; n= ?)	0.172 mg/m^3 (nose only)	2 weeks	BAChE; RAChE	>0.172 mg/m^3	Lan86
	rat (F344; ?)	0, 0.014, 0.072 mg/m^3 whole body)	2 weeks	BAChE; RAChE	>0.072 mg/m^3	Str87
	rat (Wistar; ?)	0, 10, 94, 388 mg/m^3 (whole body)	2 weeks	BAChE; RAChE	LOAEL: 10 mg/m^3	Ken88
	rat (F344; n=15/sex/group)	0, 0.07, 0.14, 0.28 mg/m^3 (nose only)	13 weeks	BAChE; RAChE	>0.28 mg/m^3	New88b
	rat (F344; n=10/sex/group)	0, 0.072, 0.143, 0.287 mg/m^3 (nose only)	13 weeks	BAChE; RAChE	>0.287 mg/m^3	Cor89
dermal	rat (F344; ?)	0, 1, 10, 100, 500 mg/kg bw/d	4 days	RAChE	1 mg/kg	Cal88, Cal89
	rat (F344; ?)	0, 0.1, 0.5 or 5 mg/kg bw/d	21 days	BAChE; RAChE	5 mg/kg	Cal88, Cal89

oral	rat (Sprague-Dawley; n=20/sex/group)	0, 0.03, 0.15, 0.75 mg/kg bw/d	6 months	BAChE RAChE	>0.75 mg/kg 0.15 mg/kg	Cou71
	rat (Sprague-Dawley; n=20/sex/group)	0, 0.025, 0.5, 10 mg/kg bw/d	13 weeks	red blood cell count; haemoglobin level	LOEL: 10 mg/kg	Cro85
	rat (F344; ?)	0, 0.1, 1, 5, 15 mg/kg bw/d	13 weeks	BAChE RAChE	1 mg/kg 0.1 mg/kg	Sza88
	rat (Long-Evans; n=10/sex/group)	0, 1, 3, 10 mg/kg bw/d	4 weeks	BAChE RAChE NTE cognitive function	1 mg/kg LOAEL:1 mg/kg >10 mg/kg 3 mg/kg	Mat96
	rat (F344; n=10/sex/group)	0, 0.1, 1, 5, 15 mg/kg bw/d	13 weeks	neurotoxicity (FOB, neuropathology)	>15 mg/kg	Sha93
	mouse (CD-1; n=40/sex/group)	0 or 2.7 /3.4 (M/F[d]) mg/kg bw/d	4 weeks	BAChE RAChE	>2.7/3.4 mg/kg LOAEL: 2.7/3.4 mg/kg	Dav85
	mouse (CD-1; n=12/sex/group)	0, 1, 10, 40, 90, 200 mg/kg bw/d	13 weeks	BAChE; RAChE	1 mg/kg	Cro87
	dog (beagle; n=4/sex/group)	0, 0.5, 1.0, 2.0 mg/kg bw/d	6 weeks	BAChE RAChE PTAChE	1.0 mg/kg LOAEL:0.5 mg/kg 1.0 mg/kg	Mar01
	dog (beagle; n=4/sex/group)	0, 0.01, 0.22, 5 mg/kg bw/d	13 weeks	BAChE RAChE	0.22 mg/kg 0.01 mg/kg	Har89
	dog (beagle; n=3/sex/group)	0, 0.01, 0.03, 0.1, 1, 3 mg/kg bw/d	1 year	BAChE RAChE	1 mg/kg 0.1 mg/kg	McC74
	dog (beagle; n=4/sex/group)	0, 0.01, 0.03, 0.1, 1, 3 mg/kg bw/d	2 years	BAChE RAChE	1 mg/kg 0.1 mg/kg	McC74
	monkey (rhesus; n=1-2/sex/group)	0, 0.08, 0.4, 2 mg/kg bw/d	6 months	BAChE RAChE	>2 mg/kg 0.08 mg/kg	Cou71
	chicken (Leghorn; n=15)	10 mg/kg bw/d	20 days	lymphocyte NTE, brain NTE, BAChE	LOAEL: 10 mg/kg	Ric93

subcutaneous	rat (Long-Evans; male; n=7-8/group)	0, 15, 30, 60 mg/kg bw/d	20 weeks	cognitive function	LOAEL: 15 mg/kg	Bus94

[a] BAChE=brain AchE; RAChE= red blood cell AChE; PTAChE= peripheral tissues AChE.

In summary, inhibition of cholinesterases occurs at dose levels that are lower than those causing other toxic effects. In all species, plasma ChE and red blood cell AChE are more sensitive for inhibition by chlorpyrifos than brain AChE. Monkeys and dogs seem to be more sensitive and mice less sensitive for effects on cholinesterases than rats.

Chronic toxicity and carcinogenicity

Groups of rats (Sherman; n=25/sex/group) were fed chlorpyrifos (purity: 97%) at doses equivalent to 0, 0.01, 0.03, 0.1, 1.0, or 3.0 mg/kg bw/day, for 2 years. No treatment-related mortality, signs of toxicity, or changes in food consumption, body weights, or haematological or clinical chemical parameters were observed. No differences were found in absolute and relative organ weights or upon gross and microscopic examination of tissues when control and treated rats were compared. The numbers and types of tumours were similar for treated and control rats. No further details were provided. Plasma ChE and red blood cell AChE activities were significantly decreased at 1.0 and 3.0 mg/kg bw/day in both sexes, with inhibitions ranging from 53 to 66% for plasma ChE and 65 to 86% for red blood cell AChE. At termination, brain AChE activity was significantly inhibited at 3.0 mg/kg bw/day (44% in males and 43% in females). At 1.0 mg/kg bw, brain AChE was inhibited by 7% in females and 10% in males, but these effects were not considered to be of biological significance. The NOAELs for inhibition of red blood cell and brain AChE were 0.1 and 1.0 mg/kg bw, respectively (McC74). This study was criticised and found to be inadequate for assessing long-term effects and carcinogenicity, as there were shortcomings in data collection and in reporting of gross and microscopic examination (FAO00).

In a later conducted 2-year study, in compliance with GLP standards, Fischer rats (n=60/sex/group) received diets containing chlorpyrifos (purity: 98.5%) at concentrations equivalent to 0, 0.05, 0.1, 1, or 10 mg/kg bw/day. At 10 mg/kg bw/day, body weight gain was reduced relative to controls in the absence of reduced food consumption, and an increase in the weight of adrenal glands was seen, characterised microscopically by fatty vacuolation. Red blood cell count,

haemoglobin, total protein, and globulin concentrations were decreased, and platelet counts increased. Effects in males were more pronounced than in females. There was no increase in the incidence of any type of tumour. At 24 months, plasma ChE, red blood cell AChE, and brain AChE activities were inhibited by approximately 82%, 25%, and 57% respectively. At 1 mg/kg bw/day, the only effects attributable to treatment were inhibition in plasma ChE (by 60%) and in red blood cell AChE (by 15%). The latter was not statistically significant. Brain AChE activity remained unaffected at this level. The NOAEL for inhibition of red blood cell AChE was 0.1 mg/kg bw/day, and for inhibition of brain AChE 1 mg/kg bw (You88).

In a third 2-year study, conducted according to accepted test guidelines and GLP, Fischer rats (n=60/sex/group) were fed diets containing chlorpyrifos (purity: 96%) at concentrations equivalent to 0.012, 0.3, or 6 mg/kg bw/day. Mortality rates and the incidences of clinical signs or palpable masses were not affected by the treatment. Body weights were slightly reduced in the high-dose group. A variety of non-neoplastic and neoplastic lesions were recorded, but the incidence was within the normal range and did not show statistically significant dose-response relationships. At 6 mg/kg bw/day, plasma ChE was inhibited by 95% and 96%, red blood cell AChE by 34 and 18%, and brain AChE by 58% and 61%, in males and females, respectively, at termination of the study. At 0.3 mg/kg bw, plasma ChE activity was still significantly reduced (by 36%), but red blood cell AChE inhibition (by 17% in males and 11% in females) was not statistically significantly different. Brain AChE was not affected at this level. The NOAEL for inhibition of brain AChE was 0.3 mg/kg bw and of red blood cell AChE 0.012 mg/kg bw (Cro88).

CD-1 mice (n=56/sex/group) were fed doses of chlorpyrifos (purity: 99.6%) equivalent to 0, 0.075, 0.75, or 2.25 mg/kg bw/day, for 105 weeks. No unusual behavioural changes were observed. Absolute and relative organ weights varied unrelatedly to dose. The relative liver weights for both male and female mice were significantly decreased at the low and mid dose, but not at the high dose. Microscopic examination revealed a significant difference between control and mid-dose males for the incidence of hyperplastic nodules of the liver. There was also a significant increase in the incidence of spindle cell hyperplasia of the adrenal gland for male mice at the low- and mid-dose levels, and in female mice at the low dose only. Lesions observed in the lung included a significant increase of alveologenic adenomas in mid-dose male mice only. The histological changes identified appear to be spontaneous in nature and without a demonstrated dose response. Plasma ChE and red blood cell AChE activities, measured in animals

in the high-dose group at week 1 of the study, were inhibited by 90% and 50%, respectively. No other ChE measurements were conducted in any group at any time point. The NOAEL for systemic effects was 2.25 mg/kg bw/day (War80). Because of an inadequate data collection on ChE activities, the committee cannot establish NOAELs/LOAELs for ChE inhibition.

In another carcinogenicity study in mice, conducted according to accepted test guidelines and GLP, CD-1 mice (n=64/sex/group) were fed chlorpyrifos (purity: 96%) at concentrations equivalent to 0, 0.9, 8.8, or 45 mg/kg bw in males and 0, 0.9, 9.8, or 48 mg/kg bw in females, for 80-82 weeks. At the high dose, clinical signs (ocular opacities, lachrymation) and reductions in body weight were observed. Microscopic examination revealed increased incidences of keratitis and hepatocyte fatty vacuolation. In addition, an increased incidence of lung nodules was seen, which were characterised as bronchial-alveolar adenomas or carcinomas by microscopic examination. However, the increase of these lesions was not statistically significant. No statistically significant changes in the incidence of other neoplasms in treated animals, compared with controls, were found. In the male and female animals of the high-dose group, plasma ChE, red blood cell AChE, and brain AChE activities were inhibited by ca. 98%, 23% (not statistically significant), and 80-85%, respectively, at week 42, and 98%, ca. 30%, and ca. 85%, respectively, at week 78. In the other dose groups, there was a considerable intra-group variation. In the mid-dose group, reductions were observed in plasma ChE activities in males and females at week 42 and 78 (ca. 95% inhibition; $p<0.001$), in red blood cell AChE activities in females at week 42 (41% inhibition; $p<0.01$) and in males at week 78 (29% inhibition; $p<0.05$), and in brain AChE in females at week 42 (46% inhibition; $p<0.05$) and in males at week 42 (43% inhibition; not significant) and week 78 (47% inhibition; not significant). In the low-dose group, only plasma ChE activities were significantly inhibited (45-50%). The NOAEL for red blood cell and brain AChE was 0.9 mg/kg bw/day (Gur91). The results of chronic toxicity/carcinogenicity studies in rats, mice are summarised in Table 3.

Table 3 Summary of chronic oral toxicity/carcinogenicity studies for chlorpyrifos.

species (strain ; number; sex)	dose levels (mg/kg bw/d)	exposure duration	critical effect[a]	NOAEL (mg/kg bw/d)	reference
rat (Sherman; n=5/sex/group)	0, 0.01, 0.03, 0.1, 1, 3	2 years	BAChE RAChE	1 0.1	McC74
rat (Fischer; n=60/sex/group)	0, 0.012, 0.3, 6	2 years	BAChE RAChE	0.3 0.012	Cro88

rat (Fischer; n=60/sex/group)	0, 0.05, 0.1, 1, 10	2 years	BAChE RAChE	1 0.1	Yon88
mouse (CD-1; n=56/sex/group)	0, 0.075, 0.75 or 2.25	2 years	systemic effects	2.25	Wa80
mouse (CD-1; n=64/sex/group)	0, 0.9, 8.8/9.8, 45/48 (males/ females)	78 weeks	BAChE; RAChE	0.9	Gur91

[a] BAChE= brain blood cell AChE; RAChE= red blood cell AchE.

Mutagenicity and genotoxicity

Mutagenicity assays comprise tests for the detection of gene mutations in bacteria, yeast, and mammalian cells (*in vitro)* and cytogenicity (*in vitro* and *in vivo*) and other genotoxicity assays (*in vitro*).

- *In vitro* tests:
  - Gene mutation assays. Tests for reverse mutations in 5 strains of *S. typhimurium* were negative at concentrations up to 30,000 µg/plate both with and without metabolic activation by a rat liver microsomal S9 preparation (Gol95, Poo77, Sim77). Negative results were also found in *E. coli* WP2 (concentration not specified) (Poo77). Chlorpyrifos (purity: 97%) did not induce gene mutations in the *hprt* forward gene mutation assay in cultured Chinese hamster ovary cells (CHO) when tested at concentrations of 17.5 µg/mL in the absence or presence of S9 activation (Gol95). However, a farm-grade formulation containing 20% chlorpyrifos (no further specification) induced sex-linked recessive lethal mutations in *D. melanogaster* (Pat92).
  - Cytogenicity assays. A test for the induction of sister-chromatid exchanges (SCE) in cultured human lymphocytes showed unequivocal results at doses of 2 and 20 µg/mL, in the absence of metabolic activation (Sob82). Negative SCE results were seen in cultured CHO cells at concentrations up to 100 µg/mL without metabolic activation. At this concentration, the frequency of chromosome aberrations also remained unaffected (Mus84). In another study, an increase in chromosome aberration rate was observed in chlorpyrifos-treated cultured mouse spleen cells at 4 µg/mL, without metabolic activation (Ame92). However, no increased frequency in chromosome aberrations was observed in rat lymphocytes at concentrations up to 167 µg/mL, the highest attainable level, with and without metabolic activation (Gol95).

- Other genotoxicity assays. Chlorpyrifos did not induce mitotic recombination in *S. cerevisae* (Poo77). The chemical did not increase unscheduled DNA synthesis in primary rat hepatocytes at doses up to the highest attainable level of 35 µg/mL (Gol95).

- *In vivo* tests:

CD-1 mice did not show an increased incidence of micronuclei in polychromatic erythrocytes when treated with oral doses of chlorpyrifos (purity: 97%) of 0, 7, 22, or 70 mg/kg bw or of 90 mg/kg bw, corresponding to 0.8 LD_{50} in a subsequent test (Gol95). In an earlier study, the test was positive in bone marrow cells of mice receiving 2 intraperitoneal injections of 45 mg/kg bw of chlorpyrifos (purity: not given) over a period of one week (Ame82). A positive result was also reported in bone marrow of mice treated with a single intraperitoneal dose of 30.5 mg/kg bw, corresponding to 0.5 LD_{50} (Ben89). Alkylation of DNA has been reported in mice following intraperitoneal administration of chlorpyrifos (Mos83). A statistically significant, dose-related increase in mean comet tail length indicating DNA damage was seen in peripheral blood leukocytes 24 hours after treating male Swiss mice (n=5/group) with single (gavage) oral doses ranging from 0.28 to 8.96 mg/kg bw. Mean tail lengths were gradually decreased in all dose groups, but still statistically significantly increased at doses $\geq$ 56 mg/kg bw at 48 hours post-treatment and at 4.48 and 8.96 mg/kg bw at 72 hours post-treatment. At 96 hours post-treatment, they had returned to control levels indicating repair of damaged DNA (Rah02).

In conclusion, chlorpyrofos induced clastogenic and DNA-damaging effects in some of the tests. This may be due to the test system used (e.g., route of administration) and the type of test material used (e.g., the purity of the compound).

Reproduction toxicity

In a 3-generation study, Sprague-Dawley rats (10 males and 20 females/group) were given chlorpyrifos (purity: not given) in the diet equivalent to 0, 0.03, 0.1, or 0.3 mg/kg bw/day for the first generation, and 0, 0.1, 0.3, or 1 mg/kg bw /day for the second and third generations. F0 animals were fed test diets from 60 days prior to mating and offspring were fed test diets from weaning. F2b parent animals were continued on test diet throughout the breeding period, except that females were placed on normal diet during organogenesis. No clinical signs of

toxicity were observed in the parents or the offspring (2 litters per generation). Parental body weights and food consumption were not affected. The fertility, gestation, and lactation indices were comparable between treated groups and the controls (20 males and 40 females). However, the viability index was decreased at 1 mg/kg bw/day. According to the authors, this effect was not clearly treatment related. Red blood cell AChE activity, measured in F2 animals only, was significantly reduced at 0.3 mg/kg bw/day and above. No abnormalities were observed in haematological and clinical chemical parameters in these animals. No treatment-related gross or microscopic abnormalities were recorded in F3a pups, and no treatment-related developmental effects were observed in F3b animals. Based on inhibition of red blood cell AChE, the parental NOAEL was 0.1 mg/kg bw; the NOAEL for reproduction toxicity was 1 mg/kg bw/day (the highest dose tested) (Tho71). The committee considered the NOAEL for reproduction toxicity of 1 mg/kg bw/day questionable, because of the equivocal result of the viability index.

To supplement the finding of the equivocal decrease in viability index at 1 mg/kg bw/day in the aforementioned study, a subsequent 2-generation study was performed in which Sprague-Dawley rats (n=30/sex/group) were given chlorpyrifos (purity: 96.6-99%) in the diet at doses of 0, 0.5, 0.8, or 1.2 mg/kg bw/d for 135 days, and then bred to produce F1 litters. On lactation day 21, 30 pups of each sex were randomly selected, dosed in the same way as the F0 animals for 120 days, and then bred to produce the F2 litters. The F2 pups were weaned on lactation day 21. The F0 and F1 matings were in a 1:1 ration, with pairing for 5 days, a 7-day rest, and a pairing with a different male for a further 5 days. No clinical signs were observed in the parental animals of either generation. In the F0 animals, body weight and food intake were not affected. In the high-dose group, there were no effects on mean fertility index or length of gestation, but in the 2 lowest dose groups, reductions were seen in both endpoints. Litter sizes, survival indices during lactation, and pup weights were all comparable at all doses. In the F1 animals, decreased body weights were seen in the male animals of the high-dose group on days 160-182 after cohabitation. Female body weights were slightly, sporadically increased when compared with controls but were not affected during lactation. The mean fertility index in the treated groups exceeded the control values; litter size, survival index during lactation, and pup weight were comparable among all groups. Data on cholinesterase activities were not reported (Die83).

In a third 2-generation study, groups of male and female rats (CrL:COBS CD (SD) BR; F0: n=28/sex/group; F1: n=24/sex/group) were given technical grade

chlorpyrifos (purity: 95.8%) in their diets at doses equivalent to 0.1-0.2, 0.5-0.9, and 2.5-4.6 mg/kg bw /day in F0 animals and to 0.1-0.3, 0.7-1.6, and 3.3-8.1 mg/kg bw/day in F1 animals. These doses were selected from a preliminary one-generation study in which a dietary dose of chlorpyrifos of 50 mg/kg feed (50 ppm: i.e., the high dose in the definite 2-generation study) induced inhibition of plasma ChE activity of 86% in adult females and of about 60% in weanling males and females, of red blood cell AChE of 70% in adult females and of about 50% in weanlings, and of brain ChE of 38% in adult females when compared with those in controls). However, in the 2-generation study, cholinesterase activity was not measured. Under the conditions of this study, no adverse treatment-related effects on any of the endpoints investigated were observed at any dose in any of the groups (Jam88).

Finally, in a fourth 2-generation study, Sprague-Dawley rats (n=30/sex/group) received chlorpyrifos (purity: 98%) in the diets at concentrations equivalent to 0, 0.1, 1.0, or 5.0 mg/kg bw/day. Exposure to the treated diets was continuous throughout the duration of the study. After 10 weeks on test diets, F0 parental rats were mated to produce F1 litters. After 12 weeks of treatment following weaning, F1 adults were bred to produce the F2 litters. At 5.0 mg/kg bw/day, body weights and body weight gain were slightly reduced in F1 and F2 parents. At termination, some 19-21 weeks after starting the treatment, F1 and F2 parental effects included decreased plasma ChE and red blood cell AChE activities at 1.0 mg/kg bw/day, and decreased plasma ChE, red blood cell AChE, and brain AChE activities at 5.0 mg/kg bw/day. Microscopic examination revealed alterations of the adrenal *zona fasciculata* at 5.0 mg/kg bw/day, characterised as slight vacuolation consistent with fatty changes. No effects were observed on histological examination of reproductive tissues, or on fertility, length of gestation, time to mating, or litter size in either generation at any dose level. No neonatal effects were observed at 0.1 and 1.0 mg/kg bw/day in the F1 and F2 litters. Parental toxicity at the high dose was accompanied by decreased pup body weight and increased pup mortality in the F1 litters only. The NOAEL for parental effects was 0.1 mg/kg bw/day, based on inhibition of red blood cell AChE. The NOAEL for reproduction toxicity was 1 mg/kg bw/day (Bre96).

Chlorpyrifos was also evaluated for developmental toxicity in rats, mice, and rabbits and for developmental neurotoxicity in rats.

In a rat study, groups of pregnant Fischer 344 rats (n=31-33/group) were given chlorpyrifos (purity: 96.6%) by gavage in corn oil at doses equivalent to 0, 0.1, 3, or 15 mg/kg bw/day, on days 6-15 of gestation. Blood samples for

cholinesterase determinations were collected on day 15, and fetuses were surgically removed and maternal animals sacrificed on day 21. Maternal effects at the highest dose were cholinergic signs (tremors and excessive salivation), decreased body weights and body weight gain and inhibition of plasma ChE (by 97%) and red blood cell AChE (by 79%). The only maternal effects observed at 3 mg/kg bw/day were depressions of plasma ChE (by 89%) and red blood cell AChE (by 74%). No effects on absolute and relative liver weights were noted. No abnormalities were seen in the number of litters, implantation sites, and live and resorbed fetuses. Mean fetal body weight and crown-rump length did not show changes in any of the treated groups compared to the controls, and no treatment-related teratogenicity was observed. The maternal NOAEL was 0.1 mg/kg bw/day and the developmental NOAEL at least 15 mg/kg bw (Bre95, Oue83). In another study, groups of CD Sprague-Dawley rats (n=32/group) received chlorpyrifos (purity: 96%) in maize oil at oral (gavage) doses equivalent to 0, 0.5, 2.5, or 15 mg/kg bw/day, on days 6-15 of gestation. Blood samples were collected on day 15 and necropsy took place on day 20. Maternal effects observed at 15 mg/kg bw/day were cholinergic signs (tremors), decreases in food consumption, body weight gain, and plasma ChE activity. Red blood cell or brain AChE activities were not determined. At 15 mg/kg bw/day, the mean number of live male fetuses was decreased and post-implantation loss was statistically significantly elevated. Mean fetal weight and mean crown-rump length were statistically significantly increased. No treatment-related teratological effects (visceral organs, skeleton, abdomen) were noted at any dose level. The NOAEL for systemic maternal toxicity and for developmental toxicity was 2.5 mg/kg bw/day (Rub87a).

Groups of pregnant CF-1 mice (n= 40-47/group) were given 0, 1, 10, or 25 mg/kg bw/day of chlorpyrifos (purity: 96.8%) by gavage, on days 6 through 15 of gestation. Maternal blood samples were collected on gestational days 6, 10, and 15, and animals were sacrificed at day 18. Maternal effects included mortality, cholinergic signs (tremors, excessive salivation), decreased body weight gain, and decreased plasma ChE (by 98%) and red blood cell AChE (by 57%) activity at 25 mg/kg bw/day, and cholinergic signs and decreased plasma ChE (by 96%) and red blood cell AChE (by 43%) activity at 10 mg/kg bw/day. No effects were observed on number of pregnant animals with litters, number of implantation sites per dam, and number of fetuses per litter. Fetal body weight and crown-rump length were reduced at 25 mg/kg bw and fetal tissue homogenate ChE was significantly reduced at 10 and 25 mg/kg bw. A statistically significant increased incidence of delayed ossification was seen in

fetuses at 25 mg/kg bw and an increased incidence of exencephaly was observed at the lowest dose, but not at 10 or 25 mg/kg bw/day. The study was repeated with doses of 0, 0.1, 1, or 10 mg/kg bw/day of chlorpyrifos on groups of 35-41 pregnant mice per dose level. No evidence of a teratogenic response was found. However, plasma ChE and red blood cell AChE activities were significantly decreased among maternal mice given 1 and 10 mg/kg bw/day, but not at the lowest dose. Fetal tissue homogenate ChE was only inhibited at 10 mg/kg bw/day. Based on inhibition of cholinesterases, the maternal NOAEL was 0.1 mg/kg bw/day and the developmental NOAEL 1 mg/kg bw/day (Dea80).

In a developmental toxicity study in rabbits, groups of New Zealand white rabbits (n=14/group) were given chlorpyrifos (purity: 96.1%) in maize oil by gavage at doses of 0, 1, 9, 81, or 140 mg/kg bw/day, on days 7-19 of gestation. Blood samples were collected after at least 10 days of dosing, and the does were sacrificed on day 29. Maternal effects noted at 140 mg/kg bw/day included reduced food consumption, body weight loss, and a 72% reduction of plasma ChE activity. The inhibition of plasma ChE was dose related and was still 56% at the lowest dose level. Brain or red blood cell AChE activity was not determined. Statistically significant increases in post-implantation loss were observed at 9 and 140 mg/kg bw/day, but not at 81 mg/kg bw/day. Slightly decreased fetal weights and crown-rump lengths, and an increased incidence of fetuses with unossified fifth sternebra and/or xiphisternum were observed at the highest dose level. The maternal NOAEL for systemic toxicity was 81 mg/kg bw and the maternal LOEL 1 mg/kg bw, based on inhibition of plasma ChE. The developmental NOAEL was 81 mg/kg bw/day (Rub87b).

Several studies have been reported on developmental neurotoxicity in rats. Pregnant Sprague-Dawley rats (n=5/group) were injected subcutaneously with either peanut oil or chlorpyrifos (purity: 98%) at 200 mg/kg bw as a single dose on gestation day 12, and then sacrificed on either day 16 or day 20 of gestation or on post-natal day 3, for measurement of maternal and developmental toxicity. Maternal effects noted were decreased body weights on day 3 after treatment, inhibition of brain AChE (82-88%) at all 3 time points after dosing, and reduction of brain muscarinic receptor binding (30-32%) on day 20 of gestation or post-natal day 3. At days 16 and 20 of gestation, chlorpyrifos did not alter fetal body and brain weights, but fetal brain AChE activity was inhibited by 42-44%. At post-natal day 3, inhibition of pup brain AChE was still 30%. Pup brain muscarinic receptor binding was inhibited by 16 and 11%, respectively, on day 20 of gestation or on post-natal day 3. No parameters for teratogenicity were studied (Cha95). In a follow-up study, rats were given subcutaneous doses of

6.25, 12.5, or 25 mg/kg bw/day, on days 12-19 of gestation, and then sacrificed on day 20 of gestation or on post-natal day 3. No maternal toxicity was seen at any dose, but brain AChE activity on day 20 of gestation was inhibited by 75% and 90% at 6.25 and 25 mg/kg bw/day, respectively. At 25 mg/kg bw/day, pup weight on post-natal day 1 was significantly reduced. Fetal brain AChE activity on day 20 of gestation was inhibited by 40% and 60% at 6.25 and 25 mg/kg bw/day, respectively. Muscarinic receptor binding was reduced in a dose-related manner in both maternal and fetal brain. These neurochemical effects were more severe in dams than in the developing fetuses and were more severe following repeated exposure compared to the equivalent acute dose (Cha96).

In a rat development neurotoxicity study, pregnant Sprague-Dawley rats (n=24-25/ group) were given chlorpyrifos (purity: 99.8%) in corn oil at oral (gavage) doses of 0, 0.3, 1, or 5 mg/kg bw/day, from gestation day 6 through lactation day 10. F1 generation litters (n=8-20/sex/group) were observed up to post-natal day 91. An additional 5 pregnant rats per dose level received chlorpyrifos on gestation day 6-20, and were used to obtain brain, plasma, and red blood cell samples for cholinesterase determinations. Effects in dams given doses of chlorpyrifos of 5 mg/kg bw/day were cholinergic toxicity (muscle fasciculations, hyperpnoea, and hyperreactivity), a treatment-related reduced body weight gain toward the end of gestation and the beginning of lactation (not statistically significant), reduced food consumption at the beginning of lactation, and marked inhibition of brain AChE and plasma ChE (approximately 90%), and of red blood cell AChE (100%) on gestation day 20. At 1.0 mg/kg bw/day, brain and red blood cell AChE of the dams were depressed by 18% and 84%, respectively, and plasma ChE by 69%. At the low dose, brain AChE activity was not affected, while red blood cell AChE and plasma ChE were still depressed by 41% and 43%, respectively. Developmental parameters in dams, i.e., duration of gestation, number of implantations per dam, and mean litter size on post-natal day 0 were comparable across the groups. A statistically significant increase in pup mortality on post-natal day 0 and post-natal days 1-4, lower pup body weights on post-natal days 0 and 4, and a decreased viability index were seen at 5 mg/kg bw/day. By post-natal day 65, the weights of high-dose female offspring were comparable with controls, while the weights in male offspring remained lower. At 5 mg/kg bw/day, increased pinna detachment, preputial separation, and vaginal opening were also observed. Post-natal day-11 brain measurements revealed decreased absolute brain weights, increased relative brain weights, and decreased brain layer thickness (e.g., of the parietal cortex, and the hippocampus) in high-dose male and female offspring. The differences in brain

layer thickness disappeared after correction for brain weight. In post-natal day-65 rats, no brain effects were observed, except a decrease in the parietal cortex of adult females in the mid- and high-dose groups, compared with controls. However, after correction for brain weight, the high-dose female adult group was not statistically significantly different from the control group anymore. Cognitive functions (learning, short-term memory, and habituation in 2 different tasks) in the pups were not affected by treatment. There were no gross or microscopic lesions of the nervous system. The authors conclude that no evidence of specific toxicity of chlorpyrifos for the developing nervous system was identified and that the effects seen in high-dose offspring were a consequence of maternal toxicity, rather than a direct effect on the pups. The maternal LOAEL was <0.3 mg/kg bw, based on inhibition of red blood cell AChE and plasma ChE. The offspring NOAEL was 1 mg/kg bw/day (Mau00). In another study, it was demonstrated that fetal exposure from high-dose dams (5 mg/kg bw/day) caused a 57% inhibition of fetal brain AChE on gestation day 20. On post-natal day 1, pup brain AChE was inhibited by 35%, but no inhibition of brain AChE was seen anymore in pups on post-natal day 5. In dams, brain AChE activities on lactation day 1 and lactation day 5 were inhibited by 85% and 78%, respectively. No inhibition of brain or red blood cell AChE, or plasma ChE occurred in fetuses or pups from dams given 0.3 or 1.0 mg/kg bw/day. Neonatal mortality from high-dose dams between post-natal days 1 and 5 did not correlate with inhibition of neonatal brain AChE (Mat00). According to the committee, these data justify the above offspring NOAEL of 1 mg/kg bw/day.

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for chlorpyrifos in the Netherlands is 0.2 mg/m^3, 8-hour TWA, with a skin notation.

Existing occupational exposure limits for chlorpyrifos in some European countries and in the USA are summarised in the annex.

8 Assessment of health hazard

The health hazard assessment of chlorpyrifos is based to a large extent on toxicology reviews issued by the Health Effect Division of the United States EPA for reregistration eligibility and by the FAO/WHO Joint Meeting on Pesticide Residues for recommendation of an acceptable daily intake (ADI). The toxicology profile in these reviews is obtained mainly from unpublished reports

of toxicology studies conducted for registration purposes by the chemical companies manufacturing or marketing the product.

Workers can be exposed to chlorpyrifos through inhalation of vapour or aerosols or by direct skin contact with a formulation of the compound. The committee did not find data on the percentage of uptake of the compound through the lungs in humans. In rats exposed to chlorpyrifos by nose-only, 72% of the inhaled dose was absorbed. The dermal absorption of chlorpyrifos was about 2% of the applied dose (in 120 hours); in mice about 74% (in 8 hours). The extent of absorption following oral intake is at least 70% in humans and essentially 100% in the rat. Following absorption, the compound is metabolised into breakdown products (e.g., DEP, DEPT, 3,5,6 TCP), which are mainly excreted in the urine (>70% in humans and rats). There is no evidence of accumulation of the compound in any of the tissues.

Case studies in humans show that following ingestion of high doses of chlorpyrifos, nerve injury may develop, consistent with organophosphorus-induced delayed polyneuropathy (OPIDN). In a recent acute oral toxicity study with chlorpyrifos in human volunteers, the NOAEL for inhibition of red blood cell AChE activity was 1 mg/kg bw. In a short-term oral human volunteer study, ingestion of 0.014 mg/kg bw/day chlorpyrifos for 27 days did not produce statistically significant inhibition of either red blood cell AChE or plasma ChE. Red blood cell AChE activity was not inhibited following oral intake of 0.1 mg/kg bw/day for 9 days. At this dose, the mean plasma ChE was inhibited by 66%, indicating that in humans, plasma ChE is much more sensitive for inhibition by chlorpyrifos than red blood cell AChE. Results from 3 cohort studies on chlorpyrifos workers from the same manufacturing plant did not show neurobehavioural effects or changes in central and peripheral nervous system function. Mean plasma ChE inhibitions of 5-20% were found at airborne chlorpyrifos levels between 0.01 and 0.2 mg/m^3, 8-hour TWA, but the committee considers that part of the observed plasma ChE inhibition might be due to dermal absorption. No changes were found in red blood cell AChE of workers who excreted 1-year average 3,5,6 TCP concentrations in overnight urine samples up to 1.5 mg/g creatinine, corresponding with an absorbed chlorpyrifos dose of approximately 0.043 mg/kg bw/day.

In experimental animals, the compound is slightly irritating to the eyes and the skin, but has no sensitising potential. Based on the results of acute lethal toxicity studies in test animals, the committee considers the compound as toxic after inhalation and oral exposure, but unlikely to present a hazard by dermal contact. There was considerable variation in susceptibility between and within

species. Young rats were more susceptible to adverse effects compared to the adult animal after an acute exposure to chlorpyrifos. The earliest neurotoxic effect was inhibition of brain AChE activity. In rats, chlorpyrifos did not cause either neurological changes consistent with OPIDN or inhibition of neuropathy target esterase (NTE). However, in chicken, inhibition of NTE and mild delayed neuropathy was observed in a number of studies.

Systemic effects in short-term or long-term toxicity studies in rats or mice revealed ocular opacity, keratitis, fatty vacuolisation of hepatocytes and adrenals, and reduction in haemoglobin levels and erythrocyte count at high oral doses. At high subcutaneous doses, reduction in motor activity and impairment of cognitive function were observed. However, the committee considers the relevance of these findings unclear, because of the route of administration and the relatively high dose levels.

In studies with rats, mice, dogs, and monkeys, inhibition of plasma ChE, and of red blood cell AChE or brain AChE has been demonstrated. In all species examined, brain AChE has shown to be less sensitive for inhibition by chlorpyrifos than red blood cell AChE, which in turn is less sensitive for inhibition than plasma ChE. The inhalation NOAEL for inhibition of brain and red blood cell AChE was >0.28 mg/m^3 for rats (13-week study). The oral NOAEL for inhibition of brain and red blood cell AChE was >2 and 0.08 mg/kg bw/day, respectively, for monkeys (6-month study), 1 and 0.1 mg/kg bw/day, respectively, for dogs and rats (2-year studies), and 0.9 mg/kg bw/day for mice (78-week study).

Chlorpyrifos did not cause gene mutations in *in vitro* tests, but conflicting results were obtained in *in vitro* and *in vivo* tests for clastogenic effects. According to the committee, the test systems or the type of test material used may cause this discrepancy. Carcinogenicity studies in rats and mice did not show treatment-related increased incidences of neoplastic lesions. In reproduction toxicity studies in rats, treatment-related effects of chlorpyrifos mainly consisted of cholinergic signs, reduction in body weights, and of inhibition of cholinesterase activity in parental or maternal animals. Fetotoxicity was mainly characterised by reduced pup viability, occurring at doses above those causing parental or maternal toxicity. The lowest oral NOAEL for reproduction toxicity in the rat was 1 mg/kg bw/day. Developmental NOAELs ranged from 1 mg/kg bw/day in a mouse study, to 81 mg/kg bw/day in a rabbit study. There was no evidence of treatment-related malformations in any of the studies. A developmental neurotoxicity study in the rat did not show treatment-related abnormalities in the brain of offspring at the highest dose tested (5 mg/kg

bw/day). Comparative cholinesterase inhibition data for the dams and the pups showed that pups were much less sensitive for inhibition of brain or red blood cell AChE than the dams.

Based on the above data, the committee concludes that the mechanism of toxicity of chlorpyrifos in mammals is through inhibition of AChE activity in nerve tissue, occurring at dose levels that are lower than those that cause other toxic effects. Therefore, the committee identifies inhibition of brain AChE as the critical effect. In human beings, for obvious reasons, brain AChE cannot be measured. Instead, red blood cell AChE, being the same molecular target for inhibition by organophosphorus pesticides as brain AChE, is used as a surrogate for brain AChE in assessing the human health risk of exposure to chlorpyrifos (Jey94). Studies in rats, mice, dogs, and monkeys showed that red blood cell AChE is approximately 10 times more sensitive for inhibition by chlorpyrifos than brain AChE, and it may be assumed that this also is the case in humans. Chlorpyrifos did not cause inhibition of red blood cell AChE in any person involved in the short- or long-term human studies. The highest absorbed chlorpyrifos dose in a plant worker was calculated to be approximately 0.04 mg/kg bw/day. Based on a 70-kg worker and assuming 100% pulmonary absorption in the absence of dermal uptake, the tentative NOAEL for inhibition of red blood cell AChE is greater than 0.3 mg/m^3/day.

Because of the absence of a NOAEL for red blood cell AChE in humans, the committee takes the 2-year rat study as a starting point in deriving a HBROEL. The NOAEL for inhibition of brain AChE was 1 mg/kg bw/day. Since workers are exposed for 5 days a week, this NOAEL from a continuous study (i.e., 7 days/week) is adjusted by multiplying with a factor of 7/5 resulting in a no-adverse-effect level (NAEL) of 1.4 mg/kg bw. For the extrapolation to a HBROEL, a factor of 4 for allometric scaling from rats to humans, based on caloric demand, and an overall factor of 9, covering the absence of a NOAEL and inter-and intraspecies variation, are applied, resulting in a NAEL for humans of 0.04 mg/kg bw/day. Assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred value approach, a HBROEL of 0.2 mg/m^3 is recommended for chlorpyrifos.

The committee recommends a health-based occupational exposure limit for chlorpyrifos of 0.2 mg/m^3, as an 8-hour time-weighted average (TWA). Because chlorpyrifos can be absorbed through the skin in significant amounts, the committee recommends a skin notation.

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Annex

Occupational exposure limits for chlorpyrifos in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	0.2	8 h	administrative	S	SZW03
Germany						
- AGS	-	0.2	8 h		S	TRG00
- DFG MAK-Kommission	-	-				DFG02
Great Britain						
- HSE	-	0.2	8 h	OES	S	HSE02
		0.6	15 min			
Sweden	-	-				Arb02
Denmark	-	0.2	8 h		S	Swe00
USA						
- ACGIH	-	0.1[c]	8 h	TLV	S, A4[d]	ACG03b
- OSHA	-	-			S	ACG03a
- NIOSH	-	0.2	10 h	REL	S	ACG03a
	-	0.6	15 min			
European Union						
- SCOEL	-	-				EC03

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Measured as inhalable fraction of vapour and aerosol.

[d] Classified in carcinogen category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.