

Chlorpyrifos as a Possible Global Persistent Organic Pollutant

Meriel Watts, PhD
merielwatts@xtra.co.nz

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For



Pesticide Action Network North America
1611 Telegraph Ave. Suite 1200
Oakland, CA 94612, USA, Ph: +1 510-788-9020

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Executive summary

Chlorpyrifos is an organophosphate pesticide with a wide variety of crop and non-crop uses. Modelling studies indicate that chlorpyrifos meets Stockholm Convention criteria for persistence under Arctic conditions and it has been found in Arctic ice dating back to 1971. Chlorpyrifos persists in termiticide treatments using high application rates and in freshwater sediment under anaerobic conditions. Chlorpyrifos exceeds Stockholm Convention criteria for bioaccumulation with most reported values of log K_{ow} meeting or exceeding 5.0. Chlorpyrifos undergoes long-range transport and has been measured consistently in the Arctic, in ice, snow, fog, air, seawater, lake sediment, fish and vegetation. It is amongst the pollutants with the highest concentrations present in the Arctic, in excess of most legacy POPs pesticides. Chlorpyrifos is highly toxic to aquatic organisms and a potent developmental neurotoxin at low levels of exposure, below those that trigger foetal cholinesterase inhibition. Chlorpyrifos is an endocrine disrupter with anti-androgenic and oestrogenic properties and reduces serum levels of cortisol and thyroid hormone T4. Exposures in utero and in early childhood can lead to behavioural anomalies in adolescence and adulthood. Epidemiological studies in humans found delayed cognitive and psychomotor development, and reduced IQ. Chlorpyrifos has been detected in human breast milk, cervical fluid, sperm fluid, cord blood, and the meconium of newborn infants.

Chlorpyrifos is released directly to the environment when it is applied as a pesticide. Use of the substance has greatly increased since its introduction in 1965. Alternative techniques for avoiding the use of chlorpyrifos are available for all or most of its uses. These include cultural and mechanical techniques, biological controls and other chemicals. Since chlorpyrifos can move far from its sources, individual countries or regions cannot protect themselves or abate the pollution caused by it. Due to its harmful POP properties and risks related to its widespread production and use, international action is warranted to control chlorpyrifos.

Introduction

Chlorpyrifos is a currently used broad-spectrum chlorinated organophosphate insecticide. It is used on fruit, grain, nuts, vegetables, livestock, ornamentals, golf courses, buildings, and for treating wood products. It is formulated as liquid, granular, and flowable concentrates, baits, wettable powders and dusts. In agriculture chlorpyrifos is commonly used as a foliar spray, or applied directly to soil and incorporated into it before planting. It is incorporated into paint as a means of vector control. Chlorpyrifos is considered one of the most widely used insecticides, and use occurs in most regions.

Alternative techniques for avoiding the use of chlorpyrifos are available for all or most of its uses. These include ecosystem approaches to managing crop pests, such as using resistant varieties, reducing abiotic stress, building health soils, practising crop diversity, crop rotation, intercropping, optimised planting time and weed management, conserving predators, and managing crop nutrient levels to reduce insect reproduction. Biological preparations such as azadirachtin, attractant traps and lures, and biological controls including pathogens, parasites and predators, are just some of the many techniques used to control pests on which chlorpyrifos is used.

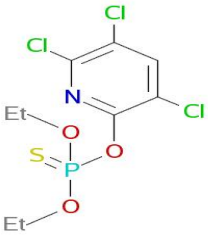
The present paper focuses solely on the information required under paragraphs 1 and 2 of Annex D of the Stockholm Convention and it is mainly based on information from the following sources: the European Food Safety Authority 2005 Review of chlorpyrifos, the United States Environmental Protection Agency Review documents, the United States National Library of Medicine's Hazardous Substances Data Bank, the University of Hertfordshire's Pesticide Properties DataBase, studies published by Dow Chemical Company, and studies published in peer reviewed journals.

1. Identification of the chemical

1.1 Name and registry numbers¹

Common name:	Chlorpyrifos
IUPAC name:	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
CAS number:	2921-88-2
Trade names include	Lorsban, Dursban, Suscon Green, Empire, Equity

1.2 Structure

Chemical structure:	
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Molecular formula: C₉H₁₁Cl₃NO₃PS

Molecular weight: 350.6

1.3 Toxicologically relevant impurities

Impurities	Concentration	Reference
Sulfotep	3 g/kg	WHO specification (maximum)
Acetone insolubles	5 g/kg	WHO specification (maximum)

1.4 Toxicologically relevant metabolites

Metabolite	Importance	Reference
3,5,6-trichloro-2-pyridinol (TCP)	primary	EFSA (2005)
3,5,6-trichloro-2-methoxypyridine (TMP)	secondary	EFSA (2005)
O-ethyl-O-(3,5,6-trichloro-2-pyridoyl) phosphorothioic acid (phosphorothioate)	not stated	EFSA (2005)
Chlorpyrifos oxon	minor	US EPA (2009)

¹ EFSA 2005; PPDB 2012

TCP is regarded as having a similar toxicity as chlorpyrifos to birds, mammals, and freshwater and estuarine/marine fish and invertebrates. Chlorpyrifos oxon may be more toxic than chlorpyrifos to these organisms (US EPA 2009).

1.5 Physico-chemical properties

Chlorpyrifos is a white to tan crystalline solid with a melting point of 41.5 - 42.5°C. It is relatively stable to hydrolysis in neutral pH and acidic aqueous solutions. Stability decreases with increasing pH. The hydrolytic stability, coupled with the aqueous photolysis half-life of 30 days, and relatively low volatilization and degradation under aerobic conditions, indicate chlorpyrifos may be persistent in the water columns of some aqueous systems with relatively long hydrological residence times (NMFS 2008).

Overview of selected physico-chemical properties

Property	Value	Remarks and Reference
Vapour pressure	1.0 x 10 ⁻³ Pa at 25 ⁰ C 2.546 x 10 ⁻³ Pa at 25 ⁰ C 6.8 x 10 ⁻⁴ Pa at 25 ⁰ C 3.35 x 10 ⁻³ Pa at 25° C 1.43 x 10 ⁻³ Pa at 20°C (99.8%)	Intermediate vapour pressure WHO (2009) EFSA (2005) Volatile – PPDB (2012)
Water solubility	0.39 mg/L at 19 ⁰ C 0.941 mg/l at 20 ⁰ C 0.588 mg/L at 20 ⁰ C 1.05 mg/l at 20°C	Low water solubility WHO (2009) EFSA (2005)
Partition coefficient octanol/water (20 ⁰ C) (25 ⁰ C)	Log K _{ow} = 5.0 at 24.5 ⁰ C Log K _{ow} = 4.7 at 20 ⁰ C Log P _{ow} = 4.7 at 20 ⁰ C Log K _{ow} = 4.96 - 5.11 Log K _{ow} = 5.2 - 5.267	WHO (2009) EFSA (2005) Gebremariam et al (2012)
Soil sorption coefficient, K _{oc}	652-30,381 L/kg	High sorption especially in high organic content soils Gebremariam et al (2012)
Aquatic sediment sorption, K _{oc}	3,000-25,565 L/kg Mean = 13,439 L/kg Median = 15,500 L/kg	Relatively higher affinity for aquatic sediment than soil Gebremariam et al (2012)
Henry's Law Constant (determines solubility of gas in liquid)	0.478 Pa x m ³ x mol ⁻¹ 2.8 x 10 ⁻⁰⁴ at 20°C	EFSA (2005) Volatile – PPDB (2012)

2. Persistence

The Annex D 1(b) criteria for persistence are:

- (i) *Evidence that the half-life of the chemical in water is greater than two months, or that its half-life in soil is greater than six months, or that its half-life in sediment is greater than six*

- months; or*
- (ii) *Evidence that the chemical is otherwise sufficiently persistent to justify its consideration within the scope of this Convention.*

An initial examination of data shown in the table below appears to indicate that chlorpyrifos does not meet the Annex D 1(b)(i) threshold criteria for persistence, with the exception of the data provided by the US EPA to POPRC on soil half life.

However, additional data indicates the following:

- (i) A number of studies show the persistence thresholds are met for both soil and water (see additional sections on soil, sediment, water and others below).
- (ii) Modelling studies indicate that persistence criteria are met under Arctic conditions.

Stockholm Convention criteria Annex D 1(b)(ii) provides for “evidence that the chemical is otherwise sufficiently persistent to justify its consideration within the scope of this Convention”. The sections below describe a series of studies that indicate chlorpyrifos meets this aspect of persistence criteria in the Convention.

Degradation data from EFSA (2005) and PPDB (2012), but with the range and conditions added in square brackets²

	Chlorpyrifos	TCP	Chlorpyrifos oxon	Reference
CAS No.	2921-88-2	6515-38-4	5598-15-2	
DT₅₀ soil lab (days) [20°C aerobic]	74 [43-111]	10-67	Not available	EFSA (2005)
DT₅₀ water sediment/whole system (days)	22-51	No data	Not available	EFSA (2005)
DT₅₀ soil field (days)	North America: 1-77 In literature: 1.3-120 Proposed value by the Rapporteur: 14	France: 8 Spain: 96	Not available	EFSA (2005)
DT₅₀ soil lab (days)	76	38.5	Not available	PPDB
DT₅₀ water sediment/whole system (days)	36.5	19.6	Not available	PPDB
DT₅₀ soil field (days)	21.0	52	Not available	PPDB
DT₅₀ soil (days)	180 ³	persistent in absence of light ⁴		US EPA

2.1 Soil persistence

There is a wide range of half-lives reported in the literature for soil persistence, ranging from a few days to 4 years, depending on application rate, ecosystem type, and various environmental factors. The dissipative half-life is significantly longer in organic soils than mineral soils (Gebremariam et al 2012).

² The data in Table 6 was provided in the Background Document to the Report “Assessment of Alternatives to Endosulfan and DDT, submitted to the POPRC Secretariat in May 2012, and were based on EFSA (2005) and the PPDB (2012).

³ UNEP-POPS-POPRC7CO-SUBM-ENDOSU-DDT-US_2-120623.En.

⁴ US EPA 2006

Racke et al (1994), of Dow Chemical Company, the manufacturer of chlorpyrifos, found half-lives of 175, 214, 230, 335, and 1576 days in five soils from different U.S. states, in laboratory dissipation studies under standard laboratory conditions (25°C, darkness, field moisture capacity). These were termiticide treatments, with application rates of 738-897 µg/gm. Termiticide application rates are much higher than in agricultural usage, and higher rates of application result in slower dissipation.

Baskaran et al (1999) determined a half-life of chlorpyrifos of 462 days in Australian red-brown soil under laboratory conditions of constant temperature at 25°C and moisture (60% maximum water holding capacity), and termiticide application rates of 1000 mg/kg. This study also found that hydrolytic breakdown of chlorpyrifos is more rapid in alkaline conditions.

In an evaluation of insecticides for soil treatment of termites in Arizona, 22% of chlorpyrifos applied at 1% active ingredient was still present in the soil 1 year after application: the initial concentration was 1420 ± 214 ppm, and the residual amount after 1 year was 315 ± 48 ppm. Where the soil was covered by a slab, the mean residual level of chlorpyrifos after 1 year was 51% of initial concentration (1601 ± 36 ppm falling to 813 ± 199 ppm), i.e. DT₅₀ > 365 days (Baker & Bellamy 2006).

Another study on the efficiency of insecticides as soil termiticides found a DT₅₀ of 8.2 months for chlorpyrifos at 0-2.5 cm depth. Chlorpyrifos was added to soil beneath a concrete slab. This was a common preconstruction technique in the USA before this use was prohibited in 2005 (Mulrooney et al 2006).

Zhong et al (2012) report that such is chlorpyrifos' persistence, that when it has been used as a soil termiticide, summertime levels in indoor air did not decline over a period of 7 years after initial application.⁵

In an experiment to determine the persistence of the toxicity of chlorpyrifos to the amphipod *Hyella curvispina* and the fish *Cnesterodon decemmaculatus*, after application in experimental soybean crops in Argentina, Mugni et al (2012) found that toxicity persistence in the runoff from the soil remained at 100% for *H. curvispina* for 42 days, then decreased slowly to 30% after 140 days. In late season applications, chlorpyrifos mortality in the soil remained at 100% until 84 days after spraying, remaining still at 80% at the end of the experiment (140 days). Early and mid season applications resulted in more rapid decay of toxicity, showing that prevailing environmental conditions alter the rate of decay of chlorpyrifos toxicity. Temperatures were lower in the late season, suggesting a decreased loss of chlorpyrifos from the soil through vaporisation and photodegradation. The persistence of chlorpyrifos toxicity to 80% after 140 days is indicative of the chemical's persistence in the soil: a half life was not identified but it is reasonable to assume it would exceed 180 days.

Chlorpyrifos dissipation from soil is faster under tropical conditions; in one study where it was applied to a mustard crop there were negligible amounts left after 70 days; half-life was 3.6-9.4 days. In other studies where chlorpyrifos has been applied to bare fields in tropical conditions, the half-life ranges from 0.6-5.4 days. Shading appears to reduce photodegradation (Chai et al 2008). It is therefore reasonable to assume that persistence will be significantly greater in the cold and often dark conditions of the Arctic.

2.2 Sediment and water

Most reported values are for soil, but it has a relatively higher affinity for aquatic sediments than soils (Gebremariam et al 2012). An environmental fate review from Dow Chemical Company (Racke 1993) gives a DT₅₀ of 150 to 200 days in anaerobic pond sediments. The Australian government review (NRA 2000) refers to pond studies that give a half-life in sediment of 200 days, exceeding the Stockholm Convention persistence criteria.

Using constructed wetlands to explore removal of chlorpyrifos and pyrethroids from agricultural runoff water in California, Budd et al (2011) demonstrated that the average DT₅₀ for chlorpyrifos in sediment under wetland and anaerobic conditions was 106 ± 54 days. Degradation of chlorpyrifos is

⁵ Zhong et al (2012) cite the following reference which has not been located: Yoshida S, Taguchi S, Hori S. 2004. Chlorpyrifos and S-421 residues in indoor air and polished rice around nine years after application for termite control. *J Soc Indoor Environ Japan* 7:7-15.

primarily due to microbial action and therefore is reduced by lower temperature, anaerobic conditions, and strong sorption to sediment. It could reasonably be expected to be much higher than this in Arctic conditions.

The persistence of chlorpyrifos under tropical conditions seems to be similar to that of endosulfan: in a water/sediment microcosm study, Laabs et al (2007) found that chlorpyrifos and endosulfan had similar DTs, and could be regarded as moderately persistent (chlorpyrifos = 36.9 days; alpha endosulfan = 20.4 days; beta endosulfan = 63.6 days).

Chlorpyrifos degradation is significantly slower in seawater than it is in fresh water. One study in California found a DT₅₀ for seawater of 49.4 days at 10°C, compared with 18.7 days in freshwater. At 20°C, the DT₅₀ for seawater was 15.2 days, so temperature has a significant effect on seawater degradation (Bondarenko et al 2004). If that effect is linear, seawater degradation in the Arctic, at 5°C, would be expected to be above the Annex D criteria of 60 days for water.

In a study in California, the persistence of chlorpyrifos in sediment from San Diego creek was found to increase significantly under anaerobic conditions: the DT₅₀ for aerobic conditions was 20.3 days but for anaerobic conditions it was 223 days, although only 57.6 in sediment from Bonita Creek (Bondarenko & Ghan 2004).

Chlorpyrifos, at a concentration of 16.2 ng/L, was found in a section of an ice core sample from the largest icecap in Eurasia – Austfonna, Svalbard, Norway – corresponding to the early to mid 1980s, indicating considerable persistence under Arctic conditions (Hermanson et al 2005).

2.3 Metabolites

The major degradate of chlorpyrifos in the environment, under most conditions, is 3,5,6-trichloro-2-pyridinol (TCP). Whilst EFSA (2005) gives a half-life for soil ranging from 10 to 96 days, the US EPA (2006) describes TCP as mobile in soils but persistent when not exposed to light, with “substantial amounts” remaining 365 days after application.

The Pesticide Properties Database (PPDB 2012) gives the water solubility of TCP as 80.9 mg/L, considerably higher than the parent compound at 1.05 mg/L.

Sardar & Kole (2005) state that TCP was at maximum value on the 30th day after application to soil in India, and decreased progressively to non-detectable on day 120. The secondary metabolite TMP was not detectable after day 120.

TCP exhibits much lower soil/water partitioning than chlorpyrifos; consequently, substantial amounts of TCP are available for runoff for longer periods than chlorpyrifos (NMFS 2008). The concentrations of TCP in sediment and water are probably comparable, and runoff occurs primarily by dissolution in runoff water rather than by adsorption to eroding soil, according to US EPA (2006).

Little information is available on other metabolites.

2.4 Degradation

Both biotic and abiotic processes contribute to the degradation of chlorpyrifos. One key process is enzymatic or clay-/metal-catalysed hydrolysis, the rate of which increases with pH and temperature. It also undergoes photolytic degradation in sunlight (Gebremariam et al 2012).

However the main route of degradation appears to be via aerobic and anaerobic metabolism. Chlorpyrifos degrades slowly in soil under both aerobic and anaerobic conditions. The main metabolite, TCP, is persistent in soils when not exposed to light (US EPA 2006). Biotic degradation is addressed in Section 4 on persistence.

Hydrolytic degradation becomes the major route of degradation in alkaline soils under low moisture conditions, but it is inhibited at high concentrations of chlorpyrifos (1000 µg/g) (Racke et al 1996).

Different sources give different values for both vapour pressure and Henry’s Law Constant, describing it variously as non-volatile, of intermediate volatility, or volatile. However, volatilization may play a

role in dissipation of chlorpyrifos under some use conditions, especially for open air uses under tropical conditions, based on a vapour pressure of 1.43 mPa (25°C) and Henry's Law constant of 2.8×10^{-04} indicating that it is volatile (PPDB 2012).

2.4.1 Hydrolysis

The main metabolites following hydrolytic degradation are TCP and O-ethyl-O-(3,5,6-trichloro-2-pyridoyl) phosphorothioic acid (phosphorothioate) (EFSA 2005).

Hydrolytic degradation – half-lives according to EFSA (2005)

pH	Temperature	Days
pH 4.7-5	25°C	63-75
pH 6.9-7	25°C	16-35
pH 8.1	25°C	25

2.4.2 Photolytic degradation

Water:

EFSA (2005) gives the following photolysis half-life values, as a function of latitude, for chlorpyrifos in water: DT_{50} =

- 15 days (mid-summer 20°N)
- 30 days (mid-summer 40°N)
- 29,200 days (mid-winter 60°N).

Photostability in water: dissipative half-life (DT_{50}) = 39.9 days was reported for natural river water under natural sunlight, presumably under European conditions (EFSA 5005).

Air:

EFSA (2005) gives the following values for photolytic degradation in air:

- direct photolysis half-life in air: DT_{50} = 1 - 2.6 days.
- photochemical oxidative degradation in air = 1.4 hours.

The Hazardous Substances Data Bank (HSDB 2012) gives the photolytic half-life in air as 4.2 hours.

EFSA (2005) gives the following values for volatilisation:

- Volatilisation from plant surfaces = 79-81% in 24 hours.
- Volatilisation from soil = 22-26% in 24 hours (temperature not given).

2.5 Factors affecting persistence

The dissipative half-life is significantly longer in organic soils than mineral soils. Hydrolysis is slower in water containing clay minerals, humate, dissolved organic matter, and suspended sediment (Gebremariam et al 2012). The addition of organic matter to soil, in the form of biochar, increased persistence from a DT_{50} of 21.3 days to 55.5 days, and to 158 ± 10.1 days in sterilised soil (Yang et al 2010).

Degradation rates of chlorpyrifos are modulated by soil pH, moisture, and temperature, as well as formulation and application rates. In one experiment degradation rates doubled with each increase of 10 °C (Racke et al 1994).

Hydrolytic breakdown of chlorpyrifos is more rapid in alkaline conditions (Baskaran et al 1999).

Shading appears to reduce photodegradation (Chai et al 2008).

Hence persistence of chlorpyrifos increases with increased soil organic matter, decreased temperature, decreased pH, and decreased ultraviolet light. Muir et al (2007) concluded that low temperatures may preserve chlorpyrifos particularly in icecaps and cold, oligotrophic lakes. It is therefore reasonable to assume that persistence will be significantly greater in the cold and often dark conditions of the Arctic

than indicated by the half-lives derived from tropical or temperate regions. Muir et al (2004), using BIOWIN and a procedure described by Gouin (2003) calculated the following relevant half-lives:

- Water $DT_{50} = 218$ days
- Soil $DT_{50} = 435$ days
- Sediment $DT_{50} = 1,414$ days

2.6 Summary of persistence

Studies show that chlorpyrifos meets the Annex D 1(b)(i) threshold for persistence in soil and sediment under some conditions:

- In a number of studies based on termiticide treatments, in which high application rates are used, half-lives have exceeded the Annex D threshold of 180 days, the highest reported being 1,576 days.
- A half-life of 223 days was reported for freshwater sediment under anaerobic conditions.
- Australia's regulatory authority gives the pond sediment half-life as 200 days.

No half-lives exceeding the threshold appear to have been reported for water: however, with the half-life in seawater of 49.4 days at 10°C, more than double its half life at 20°C (15.2 days), it is entirely feasible that the half-life in seawater under Arctic conditions would more than pass the Annex D threshold of 60 days.

Calculated half-lives for Arctic conditions all exceeded the thresholds.

Like endosulfan, chlorpyrifos is less persistent under tropical conditions characterised by faster microbial degradation, photodegradation and volatilisation (Chai et al 2008), and more persistent under temperate conditions. Its persistence increases with decreased temperature, decreased pH, and decreased light. These are the conditions of the Arctic, and therefore it is reasonable to assume that persistence will be greater under Arctic conditions. In fact sampling has demonstrated its persistence in some Arctic conditions: it has been found in ice dating back to 1971 (Ruggirello et al 2010).

There is sufficient evidence that chlorpyrifos also meets the Stockholm Convention Annex D 1(b)(ii) criterion of evidence that the chemical is persistent.

3 Bioaccumulation

The Annex D 1(c) criteria for bioaccumulation are:

- Evidence that the bio-concentration factor or bio-accumulation factor in aquatic species for the chemical is greater than 5,000 or, in the absence of such data, that the log K_{ow} is greater than 5;*
- Evidence that a chemical presents other reasons for concern, such as high bio-accumulation in other species, high toxicity or ecotoxicity; or*
- Monitoring data in biota indicating that the bio-accumulation potential of the chemical is sufficient to justify its consideration within the scope of this Convention.*

3.1 Bioconcentration and bioaccumulation factors

Bioaccumulation studies were not required for registration in the US, although there is acknowledgement that it has been detected in fish tissue (US EPA 2006), and little data on bioaccumulation appears to exist.

The US government Hazardous Substances Data Bank (HSDB 2012) identified the following studies:

- A measured log BCF value for chlorpyrifos of 2.67 was determined from a 35-day flowing-water study using mosquito fish (Veith et al 1979). Log BCF relates the bioconcentration factor to the partition coefficient Log K_{ow} .
- An experimental log BCF value of 2.50 was determined by Dow Chemical Company from a static ecosystem study using mosquito fish (Kenaga 1979).

- In a review of the environmental fate of chlorpyrifos by Dow Chemical Company, BCF values of 100-4,667 were reported in a variety of fish under field conditions. BCF values of 58-1,000 were reported in a variety of fish using flow-through aquariums (Racke 1993). According to a classification scheme, this BCF suggests the potential for bioconcentration in aquatic organisms is moderate to high, provided the compound is not metabolized by the organism (Francke et al 1994).

In addition, the 1993 review from Dow Chemical Company reported aquatic bioconcentration factors of 100-5,100 in fish (Racke 1993). Marshall & Roberts (1978), in their review of the ecotoxicology of chlorpyrifos, reported BCFs up to 6000 in fish species; however it is unclear if equilibrium was reached.

Hanson et al (1986) of US EPA reported a bioconcentration factor of 5,100 in gulf toadfish (*Opsanus beta*). Mulla et al (1973) measured a BCF range of 1200-4677 in fish in a small warm-water lake in southern California.

Log BCF values reported are 3.84 in zebrafish (El-Amrani et al 2012), and the authors of this study report other studies finding log BCFs in fish between 1.69 and 3.45.

Other measured bioconcentration factors include 1,400 in oysters (Woodburn et al 2003); 2,665 in red hybrid *Tilapia* (Thomas & Mansingh 2002); 1,700 in guppies (*Poecilia reticulata*) (Welling & de Vries 1992); and 400 in Mediterranean mussel (*Mytilus galloprovincialis*) (Serrano et al 1997).

A bioconcentration factor of 1,600 was measured in the freshwater amphipod *Gammarus pulex*. The authors of this study, based on this measurement, estimated a BCF of 4,658 in females at their maximum lipid content (lipid content varies seasonally). They also noted that the QSAR prediction using a log K_{ow} of 4.7 under-predicts BCFs compared to the measured value (Ashauer et al 2006). This gives weight to the higher values for log K_{ow} reported in the next section.

There are several studies showing bioaccumulation in plants, including duckweed *Lemna minor* L. and water lettuce *Pistia stratiotes* L. (Prasertsup & Ariyakanon 2011), and blue-green algae (Lal et al 1987). Racke (1993) reports BCFs for aquatic plants of 600-1900. Measurements taken in U.S. Western National Parks found bioaccumulation occurring in conifer needles. The concentration of chlorpyrifos in 2 year-old needles was almost double that of first year needles (yr 1 = 11.6 ng/g; yr 2 = 20.5 ng/g) (Landers et al 2008).

In a study on suspension-feeding estuarine bivalves, using a low concentration of chlorpyrifos, mean tissue absorption from particulate fractions was 23-31%, with lower uptake from dissolved/colloidal forms (7-17%). Overall $27 \pm 6\%$ chlorpyrifos from particulate absorption, and $7-17 \pm 5\%$ from colloidal absorption, was consistently accumulated into tissues, with the remainder eliminated via excretion and faecal production (Bejarano et al 2003).

3.2 Bioaccumulation potential - Log K_{ow} (octanol-water partition coefficient)

Values for log K_{ow} for chlorpyrifos vary from 4.7 to 5.11, all either close to or exceeding bioaccumulation criteria for the Stockholm Convention.

Values of log K_{ow}

Substance	log K_{ow}	Reference
Chlorpyrifos	4.7	EFSA 2005
Chlorpyrifos	5.0	PPDB 2012
chlorpyrifos at 20°C	4.96 – 5.11	Gebremariam et al 2012
chlorpyrifos	4.7; 4.82; 4.96, 5.11; 5.2; 5.27 (experimental)	Racke 1993
TCP	3.10 (estimated) 3.21 (experimental)	Background Document to the Report “Assessment of Alternatives to Endosulfan and DDT, submitted to the Secretariat in May 2012, and based on Epi-Suite
Chlorpyrifos oxon	2.89 (estimated)	Background Document to the Report “Assessment of Alternatives to Endosulfan and DDT, submitted to the Secretariat in May 2012, and based on Epi-Suite

Hence, most values for the parent compound exceed, or are very close to, the threshold value for Annex D(c) of 5.0. Even if the lowest value (4.7) is taken, this is still higher than that of lindane (3.5)⁶, a substance already added to the Stockholm Convention on Persistent Organic Pollutants and therefore deemed to have met the criteria on bioaccumulation. However, there is evidence of other values that exceeded the threshold criteria in Annex D.

3.3 Summary of bioaccumulation

Regulatory processes have not generally required bioaccumulation studies for chlorpyrifos, presumably because there has been an assumption that substances classified as organophosphates are not likely to bioaccumulate, hence there are few available studies. Nevertheless those that are available do show a significant degree of bioaccumulation in a number of species, with one review from the manufacturer, Dow Chemical Company, reporting a value of 5,100 in fish, thus exceeding the threshold value of 5,000. Additionally most reported values of log K_{ow} meet or exceed the threshold value of 5, with even the lowest value (4.7) being higher than that of the already listed POP lindane (3.5). Chlorpyrifos has been measured in fish in the Arctic, indicating potential bioaccumulation.

There is sufficient evidence that chlorpyrifos meets the Stockholm Convention Annex D 1(c) criterion of evidence that the chemical is bioaccumulative.

3.4 The PB Score

RIVM, the National Institute for Public Health, in the Netherlands has developed a tool for identifying potential POP and PBT (persistent, bioaccumulative, toxic) substances, assigning a score for persistence and bioaccumulation for many substances (Rorije et al 2011). This score is seen as a guidance based on theoretical properties derived from the chemical structure, with no experimental

⁶ UNEP/POPS/POPRC.1/10

data input. It uses the overall persistence, Pov, from the OECD Pov and LRTP Screening Tool⁷ and estimates bioaccumulation based on log Kow and other calculations. The overall PB-score varies between 0 and 2. Substances with a PB-score of ≥ 1.5 will have individual P or B-scores of at least 0.5 or higher, and therefore will be likely to comply with the thresholds for both persistence and bioaccumulation criteria. However a PB score between 1 and 1.5 can still be indicative of POPs properties, and other data needs to be considered.

The following are PB scores of some already listed POPs:

- Endosulfan = 1.35
- Lindane = 1.85
- DDT = 1.92

The PB score for chlorpyrifos is as follows:

- P = 0.819
- B = 0.609
- PB = 1.428

The PB score for chlorpyrifos is therefore higher than that for endosulfan, but lower than that for lindane.

4 Potential for long-range transport

The Annex D 1(d) criteria for potential for long-range transport are:

- Measured levels of the chemical in locations distant from the sources of its release that are of potential concern;*
- Monitoring data showing that long-range environmental transport of the chemical, with the potential for transfer to a receiving environment, may have occurred via air, water or migratory species; or*
- Environmental fate properties and/or model results that demonstrate that the chemical has a potential for long-range environmental transport through air, water or migratory species, with the potential for transfer to a receiving environment in locations distant from the sources of its release. For a chemical that migrates significantly through the air, its half-life in air should be greater than two days.*

4.1 Atmospheric half-life

Although the estimated atmospheric half-life of chlorpyrifos, given as 4.2 hrs in the HSDB and PUBCHEM⁸, indicates that chlorpyrifos is not expected to undergo long-range transport, it has been found extensively in Arctic media indicating that long-range transport is occurring and has been occurring for many years. Ruggirello et al (2010) suggest this is likely to be a result of lack of ultraviolet radiation during the polar darkness lowering concentrations of hydroxyl radicals in the air and preventing atmospheric photolysis. The lack of chlorpyrifos oxon in the Austfonna ice core further suggests low levels of oxidation of chlorpyrifos in the Arctic region. A lack of atmospheric moisture may reduce water solubility, increasing the air-water partition coefficient, K_{aw} , increasing the time chlorpyrifos remains in the gas phase and further assisting its long-range transport (Hermanson et al 2005).

4.2 Monitoring data for the Arctic and measured levels

The Arctic Monitoring and Assessment Programme (AMAP) reports that chlorpyrifos has been identified in fish, surface water, ice and fog from the Bering and Chukchi Seas, air in the eastern Canadian archipelago, and subarctic and arctic lakes in Canada (Hoferkamp et al 2010). Other studies

⁷ http://www.oecd.org/LongAbstract/0,3425,en_2649_34379_40718985_119669_1_1_1,00.html.

⁸ <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=2730#x321>.

have consistently found it in Arctic media, including air, snow, seawater, lake sediment, and vegetation.

In 1993 the BERPAC programme carried out sampling in the Bering and Chukchi marine ecosystems, identifying chlorpyrifos in seawater, marine ice, and marine fog (1-5 ng/L). It was one of the most frequently detected contaminants in the seawater (6 of 9 samples) and ice (1 integrated sample). Concentrations were highest in the marine ice (170 ng/L) and seawater (19-67 ng/L) at locations closest to the ice edge, or polynya. The polynya ecosystem is the most biologically productive zone in the Arctic marine environment, with high concentrations of Arctic marine mammals and birds and consequently important areas where northern Indigenous peoples focus their traditional hunting and fishing. During melt periods chlorpyrifos is likely to be released into the adjacent marine waters when biological spring is beginning. The authors of this study expressed the view that the measured levels of chlorpyrifos might have detrimental effects on the biota, especially aquatic organisms (Chernyak et al 1996).

Chlorpyrifos was not found in air samples in the above study, but it was found in air in a separate study, in interstitial air sampled concurrently with fog sampling in the same area, in 1993. It was also found in the fog by Rice et al (1997):

Chlorpyrifos in Arctic fog and air

Fog sample – water fraction	Air sample - vapour fraction	Air sample - particulates
0.08 ng/L	0.76 ng/L	0.08 ng/L

Fogs are common over much of the Bering and Chukchi Sea area, occurring up to 80% of the year in the Aleutian Islands, so exchanges at the interface between fog with snow, ice and seawater may be a common occurrence. Rice et al (1997) proposed that once chlorpyrifos and other contaminants are in the Arctic atmosphere, fog plays a major role in recycling them within the ecosystem.

Jantunen et al (2007) also found chlorpyrifos in Arctic air samples taken over the Labrador Sea, at 0.36-30.4 pg/m³.

In 2010 chlorpyrifos was again found in all oceanic air samples taken over the Sea of Japan, the East China Sea, and the Bering and Chukchi Seas, the concentration decreasing from Asia to the Arctic (Zhong et al 2012). Air-sea gas exchange varied from net volatilisation in east Asia (<40°N) to net deposition in the Arctic. Chlorpyrifos (along with endosulfan and dicofol) was one of the most abundant pesticides found in air and in the seawater. Chlorpyrifos levels in Arctic seawater were <1 pg/L, lower than in previous studies, perhaps reflecting lower releases following the restrictions on residential and termiticide uses in the USA. However this study showing declining gradients from Asia to the Arctic indicates that Asia continues to be a significant source of long-range transport of chlorpyrifos to the Arctic.

The upper 40m of an ice core taken from Svalbard, Norway in 1998 was analysed for the presence of a number of pesticides and metabolites. Nine compounds were not detected, including chlorpyrifos oxon. Twenty compounds were found in discontinuous layers, including many of the POPs pesticides. Eight compounds were found to have continuous profiles and these included chlorpyrifos, indicating historic deposition. Chlorpyrifos first appeared in 1972, peaked in 1980 at a concentration of 16.2 ng/L, and began to decline in the 1990s. Chlorpyrifos was not found in the 1992-1998 layer (Hermanson et al 2005).

Garbarino et al (2002) found high concentrations of chlorpyrifos (70-80 ng/L) in snow collected from sea ice at 3 sites in northwest Alaskan Arctic estuaries in the Chukchi and Beaufort Seas during 1995-96. These concentrations were higher than those of any POP or other current use pesticide tested, including endosulfan, DDT, chlordane, dieldrin, HCH, and PCBs.

According to a 2005 AMAP report, ice core segments on Holtedahlfonna in Svalbard, Norway, and compared them with segments taken at Austfonna, also in Svalbard. Back trajectory calculations show that Eurasia is the source of the chlorpyrifos 74% of the time for Austfonna, and 45% of the time for Holtedahlfonna. They found the peak flux of chlorpyrifos to be 809 pg/cm²/yr and calculated the

contaminant burden for the years 1952 - 2005 to be 776 ng, well in excess of any other pesticide (the next highest burdens were gamma-HCH at 520 ng and alpha-HCH at 402). They also calculated that the chlorpyrifos burden is higher at Austfonna by a factor of about 13. These figures indicate that chlorpyrifos has had the greatest historical impact on Svalbard of all pesticides; and the input and burden is still growing. It was first detected in 1971-80 with a comparatively low input of 64.8 pg/cm²/yr but that had increased to 808 pg/cm²/yr by 2005. Chlorpyrifos was the only currently used pesticide (including endosulfan) detected continuously in the Holtedahlfonna ice core (Ruggirello et al 2010).

In a study of U.S. western parks, chlorpyrifos was found in all parks sampled including 2 in the Alaskan Arctic, and in more than 50% of the fish samples (up to 1.2 ng/gm wet weight) (Hageman et al 2006; Landers et al 2008). It was found in snow, sediment, lichen, conifer needles and fish. The authors noted that chlorpyrifos was better accumulated in plant material than in fish. Concentrations of chlorpyrifos in vegetation ranged up to 31 ng/gm in conifers (higher than DDT, PCB, and chlordane). Levels in lichen were at or below detection limits. Chlorpyrifos and endosulfan were the most commonly detected current use pesticides. Levels found in snow were up to 2.8 ng/L (Hageman et al 2006). The study reports the flux in snow and ice cores as 204 ng/m²/yr at Lakes Burial, Matcharak and Kangilipack; and 0.5-32 ng/m²/yr at Lakes Wonder, McLeod and Kahiltna base camp. The authors stated that chlorpyrifos was identified as a potential concern because of comparatively high concentrations in vegetation, and because concentrations in sediment are increasing over time, and it is still in current use (Landers et al 2008).

Muir et al (2007) detected chlorpyrifos in water samples from 2 Canadian Arctic lakes at up to 1.6 ng/L.

4.3 Summary of potential for long-range transport

The atmospheric half-life, based on temperate conditions does not meet the annex D 1(d) threshold. However the lack of ultraviolet radiation and atmospheric moisture may account for the observed long-range transport of chlorpyrifos despite its generally assumed short atmospheric half-life.

Chlorpyrifos has been measured consistently in the Arctic, in ice, snow, fog, air, seawater, lake sediment, fish and vegetation, at significant levels such that some scientists have commented that it might have detrimental effects on the biota, especially aquatic organisms. It is amongst the pollutants with the highest concentrations present, sometimes more than endosulfan. Sampling of ice cores dates chlorpyrifos' appearance in the Arctic as at least as early as 1972 and it has been there ever since, at concentrations that are thought to have had the greatest impact of all pesticides in Svalbard, Norway. Input and burden of chlorpyrifos in the Arctic media is continuing.

There is sufficient evidence that chlorpyrifos meets the Stockholm Convention Annex D 1(d) criteria of evidence that the chemical has the potential for long-range environmental transport.

5 Adverse Effects

The Annex D 1(e) criteria for potential for adverse effects are:

- (i) *Evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of this Convention; or*
- (ii) *Toxicity or ecotoxicity data that indicate the potential for damage to human health or to the environment.*

5.1 Human toxicity

US EPA (2009a) stated that there were 126 acute incidents involving chlorpyrifos reported between 2002 and 2009, with more than 150 people affected at least 17 of which were children.

5.1.1 Genotoxicity and mutagenicity

There is evidence of chlorpyrifos' genotoxicity and mutagenicity from a number of studies, summarized below.

Genotoxicity:

- Both acute and chronic exposure to chlorpyrifos caused significantly marked DNA damage in rat tissues, namely liver, brain, kidney, and spleen, when measured 24 hour after treatment; the damage was partially repaired at 48 and 72 hours after treatment (Ojiha et al 2011).
- Chlorpyrifos caused DNA damage in fruit fly (*Drosophila melanogaster*) at 15.0 µg/L, thought to be as a result of reactive oxygen species generation (Gupta et al 2010).
- Chlorpyrifos caused increased ratio of DNA migration, as assessed by the comet assay, in human lymphocytes at 10 µM (Sandal & Yilmaz 2011).
- Cui et al (2011) found DNA strand breakage and DNA hypomethylation in mouse lymphocytes.
- Rahman et al (2002) found a significant dose-related increase in mean comet tail length, indicating DNA damage, in mice leucocytes.
- Patnaik & Tripathy (1992) concluded that the chlorpyrifos formulation Durmet was genotoxic on the basis of induction of mosaic wing spots and sex-linked recessive lethals in *Drosophila*.
- Woodruff et al (1983) found that chlorpyrifos induced a significant amount of ring-X chromosome loss in *Drosophila*.

Mutagenicity:

- Amer & Aly (1992) concluded that the chlorpyrifos formulation Dursban was mutagenic, as it induced a high percentage of metaphases with chromosomal aberrations in mouse spleen cell cultures, with sister chromatid exchanges increasing with increasing concentration of the insecticide.
- Sobti et al (1982) found significantly increased sister chromatid exchange in human lymphoid cells treated with Dursban.
- Yin et al (2009) found increased induction of micronuclei and chromosomal lesions in erythrocytes, and DNA damage in erythrocytes and liver cells of *Bufo bufo gargarizans* tadpoles exposed to the sublethal concentrations of chlorpyrifos.
- Ali et al (2008) found micronucleus induction and DNA damage in the freshwater fish *Channa punctatus* (Bloch).
- Tian & Yamauchi (2003) measured significant dose-dependent micronuclei induction in 3-day mouse embryos following maternal exposure during the early preimplantation period.
- Cui et al (2011) refer to 2 studies, published in Chinese, that found chlorpyrifos induced micronuclei in mouse bone marrow and Chinese hamster lung cells (Li et al 1993; Song et al 1997).

Chlorpyrifos also shows evidence of genotoxicity in plant cells: Dimitrov & Gadeva (1997) found statistically significant increased frequency of micronuclei in root meristem cells of smooth hawksbeard (*Crepis capillaris* L.), due to partial spindle disturbances leading to anaphase distribution of chromosomes, as a result of exposure to the chlorpyrifos formulation Dursban. The authors also refer to studies showing increased frequency of chromosomal aberrations after exposure to Dursban in fava bean (*Vicia faba*) (Amer & Farah 1983) and barley (*Hordeum vulgare*) (Kaur & Grover 1985).

In contrast, US EPA (2009b) stated that chlorpyrifos was not mutagenic in bacteria or mammalian cells but did cause slight genetic alterations in yeast and DNA damage to bacteria. The agency did not find induction of chromosome aberrations *in vitro* or clastogenic (causing breaks in chromosomes) activity in mouse micronucleus test, and did not appear to induce unscheduled DNA synthesis in rat hepatocytes.

Summary: The data is varied, but a variety of recent studies indicate that chlorpyrifos is mutagenic or genotoxic in human, rat, mouse, Chinese hamster, toad, fish, fruit fly and plant cells.

5.1.2 Carcinogenicity

Data on carcinogenicity is equivocal. US EPA (2009b) reported no evidence of carcinogenicity in animal studies, but there are a number of epidemiological studies indicating that chlorpyrifos may be carcinogenic in humans, the association being strongest for lung and rectal cancers. There are also laboratory studies referred to in the section below on endocrine disruption in which chlorpyrifos caused human breast cancer cells to proliferate. Ventura et al (2012) described chlorpyrifos as a breast cancer risk.

Epidemiological studies have found some evidence of an association between chlorpyrifos exposure and some cancers. A case-control study of men with Hodgkin's lymphoma in Canada (316 cases, 1506 controls) found a significant association with exposure to chlorpyrifos (Odds Ratio = 5.26), although the number of cases (6) was small (Karunanayake et al 2012).

Case-control studies in 3 U.S. states were pooled to evaluate the risk of non-Hodgkin's lymphoma from exposure to organophosphates. There was an elevated risk from exposure to chlorpyrifos (OR = 3.2) but there were only a small number of cases (7) (Waddell et al 2001).

A number of analyses of exposure to chlorpyrifos and increased risk of various cancers were carried out in the U.S. as part of the Agricultural Health Study, involving more than 50,000 pesticide applicators:

- Lee et al (2004), in a study of 54,383 male pesticide applicators, with a total of 2,070 malignant neoplasms, found increased risk of lung, kidney, and brain cancer although only the lung was statistically significant, with a relative risk (odds ratio) of 2.18 for lifetime-exposure days compared with non-exposed individuals. Individuals in the highest category of intensity-weighted exposure-days but not life-time exposure days, had statistically significant increases in rates of lympho-hematopoietic cancers, leukaemia and brain cancer compared with non-exposed individuals.
- A further study of the US male pesticide applicators (56,813), found a 2.7-fold increased risk of rectal cancer in the highest exposure category (Lee et al 2007).
- Alavanja et al (2003) found an increased risk (OR = 1.65) of prostate cancer amongst male applicators exposed to chlorpyrifos, but only in those with a family history of prostate cancer.
- Engel et al (2005) found a slightly increased risk (OR = 1.4) of breast cancer amongst wives of the pesticide applicators who had used chlorpyrifos themselves, and those that had not used it but whose husbands had (OR = 1.3)

Summary:

Laboratory studies have not indicated cancer, other than those showing the proliferation of human breast cancer cells. However, there are a considerable number of epidemiological studies indicating an association between exposure to chlorpyrifos and cancer, particularly lung and rectal cancer. Weaker associations have been found with non-Hodgkin's lymphoma, leukaemia, brain, prostate and breast cancer.

5.1.3 Immune toxicity

There is little information on immune toxicity of chlorpyrifos. It was not required for US registration; however US EPA (2009b) states that there is now a new data requirement for immunotoxicity, supported by reports in open literature of immunologic abnormalities in workers (Thrasher et al 1993; Gotoh et al 2001) and laboratory rats (Blakely et al 1999; Navarro et al 2001). US EPA also draws attention to the review by Galloway & Handy (2003) of the immunotoxicity of organophosphates in general.

Laboratory studies:

Navarro et al (2001) found that exposure of neonatal rats to 1 mg/kg of chlorpyrifos daily on postnatal days 1-4 had no immediate effect on T-cell mitogenic responses to concanavalin A⁹ challenge. However, once the animals reached adulthood, T-cell responses were significantly impaired. There were no deficits in basal T-cell replication rates, implying that the adverse effect of chlorpyrifos exposure was specific to mitogenic activation. Treatment during a later neonatal period (days 11-14) elicited similar deficits in adulthood. This study indicates that development exposure to chlorpyrifos results in long-term deficits in immune competence.

In the study by Blakely et al (1999), a commercial formulation of chlorpyrifos induced immune alterations in rats associated with lymphocyte subpopulations, demonstrated by the presence of normal antibody and phagocytic responses in association with reduced T-lymphocyte blastogenesis and enhanced expression of specific cell surface antigens.

A study by Rowsey & Gordon (1999) indicated that hypothermia and fever resulting from exposure to chlorpyrifos in rats is mediated by an endogenously produced cytokine, tumour necrosis factor.

In investigating the mechanisms of immunotoxicity of chlorpyrifos, Prakash et al (2009) found that it can induce apoptosis in murine thymocytes, possibly mediated through generation of reactive oxygen species.

Epidemiology:

An analysis of periodic medical examinations of 64 termite control operators using chlorpyrifos revealed severely depressed serum butyl cholinesterase activity, depressed erythrocyte acetylcholinesterase, and abnormal blood urine nitrogen and white blood cells (Gotoh et al 2001).

A study of 12 people exposed to chlorpyrifos found a high rate of atopy and antibiotic sensitivities, elevated CD26 cells (T cell activation antigen, a key modulator of immune response), and a higher rate of autoimmunity, compared with two control groups. Autoantibodies were directed toward smooth muscle, parietal cell, brush border, thyroid gland, myelin, and antinuclear antibodies (Thrasher et al 1993). In a further study of 29 people chronically exposed to chlorpyrifos, Thrasher et al (2002) again found elevated CD26 cells and increased frequency of autoantibodies, together with decreased CD5 phenotype, and decreased mitogenesis in response to phytohemagglutinin and concanavallin.

Summary:

There is evidence of immune toxicity, including effects on lymphocytes, thymocytes, T cells, tumour necrosis factor, and autoimmunity.

5.1.4 Endocrine disruption

Androgenic effects:

Chlorpyrifos is described by Hodgson & Rose (2008) in a mini review as a 'potent inhibitor' of human liver cytochrome P450-dependent (CYP450) metabolism of testosterone (Usmani et al 2003) and oestradiol (Usmani et al 2006), thought to be as a result of the interaction of highly reactive sulphur, released during the oxidative desulphuration reaction, with the haeme iron of CYP450. Pre-incubation of CYP2A4 with chlorpyrifos (2µM) followed by testosterone (100µM) resulted in 98% inhibition of testosterone metabolism (Usmani et al 2003).

In a study of 322 male partners in couples presenting to a Massachusetts, USA, infertility clinic, urinary levels of the metabolite TCP were associated with a dose-dependent decrease in oestradiol, with an interquartile range increase in TCP associated with a 1.36 pg/mL decline in oestradiol concentration (Meeker et al 2007). Oestradiol is important in male reproductive health, particularly in germ cell survival. The levels of TCP found in this study were comparable to those found in the Second National Report on Human Exposure to Environmental Chemicals, NHANES 1999-2000, which reported TCP in over 90% of urine samples in the US population. The authors concluded that

⁹ A substance known to induce mitosis.

this reduction is of potential public health importance on a population level because of widespread exposure.

Viswanath et al (2010) described chlorpyrifos as one of the most potent anti-androgenic compounds (along with endosulfan and piperophos) out of the 9 they tested. Chlorpyrifos significantly decreased biosynthesis of testosterone in rat Leydig cells; it decreased the expression of key steroidogenic enzymes (cytochrome P450_{scc}, 2B-HSD, and 17B-HSD), decreased steroidogenic acute regulatory (StAR) protein expression, and decreased luteinizing hormone receptor stimulated cAMP (cyclic adenosine monophosphate) production.

Oestrogenic effects:

Ventura et al (2012) described chlorpyrifos as a breast cancer risk. They found that low doses (0.05 µM) cause human oestrogen-dependent MCF-7 breast cancer cells to proliferate, mediated by the oestrogen receptor ER-alpha; but high doses (50 µM) induce a decrease in proliferation. However at 50 µM, chlorpyrifos induced cell cycle arrest; modification of cell cycle progression is a hallmark of tumour cells and is crucial in human cancer progression. Additionally chlorpyrifos at 50 µM but not lower concentrations induced increases in reactive oxygen species of 58% in MCF-7 cells and 108% in non-hormone dependent breast cancer cells MDA-MB-231. Reactive oxygen species are described as potent mutagens increasing genomic instability. Thus in this study chlorpyrifos contributed to breast cancer risk by 2 mechanisms: oestrogenic effect at low doses, and disruption of cell cycle through production of reactive oxygen species (oxidative stress) at high doses, in non-hormone dependent breast cancer cells. The lowest concentration used, 0.05 µM, was described as similar to levels found in water and soil.

Chlorpyrifos was oestrogenic in two *in vitro* assays: compared to 17beta-estradiol, chlorpyrifos (<50 µM) induced a 36% response in the cell proliferation assay and 25% response in the oestrogen receptor transactivation assay, using MCF-7 human breast cancer cells (Andersen et al 2002).

Kojima et al (2004) also found chlorpyrifos to be oestrogenic in an oestrogen receptor ER-alpha assay using Chinese hamster ovarian cells: at 10⁻⁵M, chlorpyrifos produced 27% of the agonist activity of E2 at 10⁻¹⁰M. Chlorpyrifos showed 20% of the agonist activity at a concentration of 7.5x10⁻⁶M in the ER-alpha transactivation assay (but not ER-beta).

Grünefeld & Bonefeld-Jorgensen (2004) found it to weakly increase mRNA levels in oestrogen receptor ERβ in human breast cancer MCF-7BUS cells.

Thyroid effects:

Thyroid hormones are also affected by chlorpyrifos. Thyroid hormone disruption can result in negative impacts on foetal brain development (Ghisari & Bonefeld-Jorgensen 2005).

Haviland et al (2010) found increased thyroid hormone levels and altered learning behaviour in female mice exposed to 1 and 5 mg/kg chlorpyrifos on gestational days 17-20 (similar effects were not found in males).

Oral chlorpyrifos (12.5 mg/kg) significantly reduced serum concentrations of cortisol and thyroxine (T4) in sheep (Rawlings et al 1998).

Serum T4, at 6mg/kg bw/day was reduced in pregnant mice, and their offspring once they had reached adulthood, at levels of exposure to chlorpyrifos that did not induce brain acetylcholinesterase inhibition or other signs of toxicity. It also induced alterations in the thyroid gland in both generations and adrenal glands only in the dams. Effects were more marked in male mice, and reduction in T3 was more marked in female mice (De Angelis et al 2009).

Another study demonstrated thyroid-disrupting activity of chlorpyrifos on rat cells. It stimulated the proliferation of thyroid-hormone dependent rat pituitary GH3 cells, the greatest effect being at 10⁻⁵M concentration. However, in the presence of thyroid hormone T3, it slightly but insignificantly increased proliferation at 10⁻⁵M, but inhibited it at 5 x 10⁻⁵M (Ghisari & Bonefeld-Jorgensen 2005).

These laboratory findings of effects on thyroid hormones are supported by the results of an analysis of urinary levels of TCP and thyroid hormones in the U.S. National Health and Nutrition Examination Surveys (NHANES) (Fortenberry et al 2012). An interquartile range increase in urinary TCP was associated with statistically significant increases in serum T4 of 3.8% in 12-18 year old males, and 3.5% in 18-40 year old males relative to the median T4 levels. It was also associated with decreases in thyroid stimulating hormone of 10.7% among men 18-40 years old, and 20% among men >60 years old, but with increases in the same hormone in women >60 years of age.

Other endocrine effects:

Gore (2001) showed that vaginal opening and first diestrus both occurred significantly earlier in the offspring of chlorpyrifos-treated vs. control rats (chlorpyrifos was administered in a single dose of 1 mg/kg on gestational day 16). Chlorpyrifos also caused significant increases in GnRH mRNA levels in adult offspring of treated rats. Pregnant rats received 1 mg/kg chlorpyrifos on gestational day 16.

Chlorpyrifos, at 1 μ M, had significant effects on the gene transcription of gonadotrophin-releasing hormone (GnRH) neurons, which regulate the reproductive axis, and on levels of GnRH mRNA, in hypothalamic GT1-7 cells. These effects may be mediated by the oestrogen receptors. The effect was biphasic with lower doses stimulating and higher doses inhibiting mRNA levels (Gore 2002).

Slotkin, et al (2005) found sex-selective elevated plasma cholesterol and triglycerides in adult rats exposed to 1 mg/kg chlorpyrifos on postnatal days 1-4. The effect was restricted to males, as was postprandial hyperinsulinemia in the face of normal circulating glucose levels.

Chlorpyrifos has also been observed to cause endocrine-disruption in fish. At 5-15ppb, it caused the lowering of serum cortisol, oestradiol and testosterone levels, without change to gonad somatic indices, in Nile tilapia (*Oreochromis niloticus*) (Oruc 2010). Studies on marine mussels *Mytilus galloprovincialis* showed complex interactions between chlorpyrifos and 17 β -estradiol in the digestive gland (Canesi et al 2011).

Bernabò et al (2011) exposed tadpoles of the frog *Rana dalmatina* to either 0.025 mg/L or 0.05 mg/L chlorpyrifos (both ecologically relevant doses). At 1 month of metamorphosis, 20-25% of those exposed to chlorpyrifos were classified as 'intersex' due to the presence of testicular oocytes (compared to none in the control group).

Summary:

Chlorpyrifos is an endocrine disruptor; it inhibits metabolism of testosterone and oestradiol, and testosterone synthesis. It is anti-androgenic and oestrogenic, causing breast cancer cells to grow. It reduces serum levels of cortisol and thyroid hormone T4, induces alterations in thyroid and adrenal glands and differentially affects levels of thyroid-stimulating hormones in men and women. It is a breast cancer risk through its endocrine actions. It also affects gonadotrophin-releasing hormone, causes sex-select effects on insulin and cholesterol levels and causes endocrine disruption in fish and frogs.

5.1.5 Reproductive toxicity

The California EPA (Cal EPA 2008) reported that there are some studies showing reproductive toxicity, at levels of exposure that do not cause excessive maternal toxicity, including resorptions, decreases in foetal weight and long-term effects on brain and behaviour. There were also physical abnormalities including small hind and fore limbs, lack of spinal development amongst litters treated at 0.3 mg/kg/day on gestation day 0-7 (Muto et al 1992). They describe the information on teratogenicity as equivocal.

Farag et al (2003) found chlorpyrifos to be fetotoxic and teratogenic in rats at a maternal dose of 25 mg/kg/day, a dose that also produced some maternal toxicity (depressed body weight and acetylcholinesterase activity). Foetal weight and viability were decreased; foetal death and early resorption were increased; and visceral, skeletal, and external variations also increased. Farag et al (2010) also found decreased number of live foetuses and increased number of dead foetuses at 25 mg/kg/day, along with decreased sperm motility and count when adult male mice were treated with 25 mg/kg/day 4 weeks before mating with untreated females.

A study by Tian et al (2005) indicates that chlorpyrifos is teratogenic and embryotoxic in mice at doses below those that cause maternal toxicity. Pregnant females were given a single intraperitoneal injection (80 mg/kg) of chlorpyrifos on day 10 of gestation and foetuses were evaluated on gestation day 17. No maternal toxicity was observed. There was a significant reduction in numbers of live foetuses, and increase in resorptions, compared with control litters. External and skeletal malformations were observed. Rates of cleft palate increased significantly (5.97%) versus control litters (0.97%). Similarly, the absence of thoracic vertebrae was increased and the number of caudal vertebrae was significantly decreased.

In a study on buffalo ovotoxicity, Nandi et al (2009) found that chlorpyrifos at 0.02 µg/ml reduced oocyte nuclear maturation, and observed a dose-dependent decline in viability and developmental competence of oocytes.

Epidemiology:

Sherman (1996) reported 4 cases of birth defects amongst children all exposed *in utero* to the chlorpyrifos formulation Dursban, including defects of 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 (undescended), microphallus, and labia (fused). All children had growth retardation, and 3 had hypotonia and profound mental retardation.

Residential chlorpyrifos exposure *in utero*, as measured by levels in umbilical cord blood, was associated with decreased birth weight and birth length in a New York study, and significantly poorer mental and motor development at three years of age (Whyatt et al 2004). This confirmed findings of an earlier study, of African-American women, in which high levels of prenatal exposure to chlorpyrifos correlated with reduced birth weight and body length (Perera et al 2003).

Cal EPA (2008) reports studies showing that exposure to chlorpyrifos has caused DNA damage in sperm, decreased sperm concentration and sperm motility, and decreased testosterone and oestradiol levels in men.

Chlorpyrifos has been detected in human breast milk (0.436 ug/kg; 0.363mg/L), cervical fluid (6.83 ug/kg), sperm fluid (0.50 ug/kg) (Cal EPA 2008), cord blood (Samarawickrema et al 2008), and the meconium of newborn infants (Ostrea 2002).

Summary:

Teratogenic effects observed in rats include skeletal malformations, small hind and fore limbs, lack of spinal development, absence of thoracic vertebrae and cleft palate. In humans, defects of the brain, eyes, ears, palate, teeth, heart, feet, nipples, and genitalia have been associated with gestational exposure to chlorpyrifos.

Reproductive effects in animals include decreased foetal weight and viability; increased foetal death and early resorption; decreased sperm motility and count; decline in viability and developmental competence of oocytes. In humans chlorpyrifos exposure is associated with decreased birth weight and birth length; DNA damage in sperm, and decreased sperm concentration and sperm motility. Chlorpyrifos has been found in a number of human reproductive tissues including cervical fluid, sperm fluid, cord blood, meconium, and breast milk.

5.1.6 Developmental neurotoxicity

U.S. EPA (2009b) stated that there is a growing body of studies in the open literature which suggest that gestational and early postnatal exposure of rat pups to chlorpyrifos may result in persistent alterations in animals when they reach adulthood. Cal EPA (2008) observed that typically studies conducted according to the guidelines for regulatory agencies have focused on frank malformation and not on behavioural or other developmental effects. They acknowledge that “recent studies suggest that chlorpyrifos affects relatively late events in brain development centred round the proliferation, differentiation, and functioning of glial cells, and the cells that provide metabolic support for neurons and those that guide axons to their proper targets within the developing central nervous system.”

Laboratory studies:

There are numerous studies showing the adverse effect of chlorpyrifos on the developing brain and nervous system. Only a few are reviewed here. A series of studies beginning in the early 1990s showed that foetal exposure to low levels of chlorpyrifos (e.g. 1mg/kg), below the threshold for foetal cholinesterase inhibition (2mg/kg) (Qiao et al 2002), interfere with the development of the mammalian brain and nervous system. These studies showed that it directly targets events specific to the developing brain, disrupting the cellular machinery of most phases of nervous system development, namely neural cell proliferation, differentiation and maturation, the formation and activity of synapses, and the proliferation and differential of glia (Whitney et al 1995; Slotkin et al 2006; Flaskos 2012).

Chlorpyrifos inhibits cell membrane function (Barber et al 2001), and is toxic to immature neurons and glia (Monnet-Tschudi et al 2000). Its most critical effects involve interference with the functioning of nuclear transcription factors that control cell fate, including their expression, phosphorylation and ability to bind to their DNA promoter recognition sites (Dam et al 1998; Crumpton et al 2000; Garcia et al 2001; Schuh et al 2002; Slotkin 2004).

Initially chlorpyrifos attacks the neurons that form at the earliest stages of brain and central nervous system development, reducing cell replication and differentiation (by impairing DNA transcription), reducing neuritic outgrowth including cholinergic projections (Song et al 1998; Dam et al 1999; Das & Barone 1999; Slotkin et al 2001; Qiao et al 2002, 2003; Howard et al 2005). This results in a reduction in neural connections and in cell signalling capabilities (Aldridge et al 2003; Slotkin 2004; Jameson et al 2006). This reduced signalling capacity leads to subsequent deficits in cholinergic synaptic activity and eventually to behavioural anomalies in adolescence and adulthood (Slotkin 1999, 2004; Slotkin et al 2001; Dam et al 2000; Levin et al 2001; Aldridge et al 2005; Slotkin et al 2006). One brief subtoxic dose (1 or 5 mg/kg body weight /day) can cause behavioural alterations during adolescence and adulthood (Icenogle et al 2004).

Glial cells, which develop later than neurons, are even more susceptible to chlorpyrifos (Qiao 2002; Slotkin 2004). Chlorpyrifos preferentially targets glial cells (Qiao et al 2002; Garcia et al 2002). Glial cells continue to develop during childhood, so exposures during this period can also cause developmental neurotoxicity (Slotkin 2004). In fact effects on the rat forebrain, which is rich in cholinergic projections and the development of which peaks in utero, were not as severe as effects on the cerebellum which peaks 2 weeks after birth and is noncholinergic (Campbell et al 1997; Crumpton et al 2000). This means that the postnatal development period may be even more vulnerable to the effects of chlorpyrifos than the prenatal period (Qiao et al 2002).

Other studies have demonstrated the diverse effects of chlorpyrifos on the developing brain:

- Doses of 1mg/kg, below the threshold for inhibiting cholinesterase, caused suppression of fibroblast growth factor fgf20 in the forebrain and fgf2 in the brain stem, whilst elevating fgf4 in the brain stem, in neonatal rats. The fgfs play a vital role in neuronal cell differentiation, neurite outgrowth and recovery from neural damage in the striatum and hippocampus (Slotkin et al 2007).
- In cell studies, concentrations of 0.005-0.1 mM chlorpyrifos oxon binds to the brain protein tubulin disrupting tubulin polymerization to form microtubules that transport cell components such as mitochondria to nerve axons. Disruption of tubulin has been implicated in neurodegenerative diseases such as Alzheimer's disease (Grigoryan & Lockridge 2009).
- Subtoxic doses of chlorpyrifos affects the expression of 277 genes in rat forebrain. Low doses (0.5-5mg/kg affected neuroactive ligand-receptor interaction, transmission of nerve impulse, synaptic transmission, regulation of protein metabolism, and DNA-dependent transmission (Stapleton & Chan 2009).

As animals mature damage is evident in a wide variety of brain regions, the most vulnerable being the hippocampus, resulting in behavioural abnormalities (Colborn 2006).

Effect levels in relation to human residue concentrations:

As previously stated the effects of chlorpyrifos on the developing brain have been observed at levels of 1mg/kg, well below the 2 mg/kg threshold for effects on acetylcholinesterase. Flaskos (2012), in reviewing the effects of chlorpyrifos oxon on the developing brain, noted that:

- In cell culture studies, the oxon interferes markedly with glial cell differentiation in the concentration range 1-10 μM , impairing the development of extensions by 47% after 24 hr. It also disrupts the microtubule network.
- In cultures of cortical neurons from newborn rats, at concentrations of 20-50 μM , the oxon increased apoptosis (cell death).
- In humans, foetal concentrations of chlorpyrifos commonly reach 8mg/kg (22.8 μM). The steady state ratio of oxon/chlorpyrifos varies but can be higher than 0.05 in adults. The ratio is expected to be considerably higher in developing organisms, thus levels of the oxon “in the low micromolar range in the developing human can be attainable”.

The chlorpyrifos oxon can be 1000 times more potent than chlorpyrifos in its damage to neurons, and 65% more damaging to glia (Flaskos 2012).

Epidemiology:

Newborn infants in New York, exposed *in utero* to chlorpyrifos from household use, were found to have delayed cognitive and psychomotor development. Those most exposed had significantly more attention problems, attention-deficit/hyperactivity disorder problems, and pervasive developmental disorder problems at 3 years of age (Rauh et al 2006). A second study found that these effects were independent of socio-economic factors (Lovasi et al 2011). In a separate study, as little as 4.6 pg/gm of chlorpyrifos in cord blood during gestation resulted in a drop of 1.4 percent of a child’s IQ and 2.8 percent of her/his working memory (Rauh et al 2011).

A recent study from Rauh et al (2012) demonstrates that prenatal exposure to chlorpyrifos is altering children’s brain structure, the effects being visible at least 11 years after birth. At levels observed with routine non-occupational use and below the threshold for any signs of acute exposure, they found significant abnormalities in the cerebral surface, enlargements derived from enlargements in the underlying white matter (glia). These abnormalities occurred in regions of the brain associated with attention, receptive language, social cognition, reward, emotion and inhibitory control. They also linked the abnormalities with reduced IQ. Their findings support those from laboratory studies, and previous epidemiological studies linking chlorpyrifos exposure with child cognitive impairment.

This was followed by a study demonstrating the same sex-selectivity observed in laboratory studies on rodents (Slotkin 2004; Haviland et al 2010): prenatal exposure to chlorpyrifos (as measured by residues in their cord blood) resulted in a greater decrement in working memory in male than in female children, at 7 years of age (Horton et al 2012).

Summary:

Chlorpyrifos is a potent developmental neurotoxin at low levels of exposure, below those that trigger foetal cholinesterase inhibition. This is demonstrated in numerous laboratory studies and a number of recent epidemiological studies. Exposures *in utero* and in early childhood can lead to behavioural anomalies in adolescence and adulthood. Epidemiological studies found delayed cognitive and psychomotor development, and reduced IQ.

5.2 Ecotoxicity

The US EPA (2009a) reassessment of chlorpyrifos identified concerns about acute and chronic risks to birds, mammals, terrestrial invertebrates, fish, and aquatic invertebrates. It stated there were 278 reported ecological incidents between 1974 and 2005 associated with the use of chlorpyrifos. Chlorpyrifos was the “probable” or “highly probable” causative agent of 108 out of 121 reported adverse aquatic incidents (such as fish kills, and for 70 out of 107 terrestrial incidents mainly bird and bee kills).

5.2.1 Aquatic toxicity

Toxicity to aquatic organisms is of the most relevance to the Arctic, given their position in the food chain, and the extent of aquatic contamination. Chernyak et al (1996) stated that the measured levels of chlorpyrifos might have detrimental effects on the biota, especially aquatic organisms. Using the Globally Harmonised System of Classification and Labelling, the EU has categorised chlorpyrifos as Aquatic Acute Tox 1, with the hazard phrase “H400 – very toxic to aquatic life”; and Aquatic Chronic Tox 1, with the hazard phrase “H410 – very toxic to aquatic life with long lasting effects”.

Exposure to sublethal concentrations of chlorpyrifos has caused the following effects in species of freshwater and marine fauna: ataxia, delayed maturation, growth and reproduction impairment, deformities, and depressed populations (Marshall & Roberts 1978; Jarvinen et al 1983; Odenkirchen & Eisler 1988; NMFS 2008).

Chlorpyrifos is genotoxic, causing micronucleus induction and DNA damage, in *Channa punctatus* (Ali et al 2008), and causes oxidative stress (Xing et al 2012). It can affect the immune system in fish: it caused a dose-dependent reduction in lymphocytes in Murray cod (Harford et al 2005), altered expression of cytokines in Chinook Salmon (Eder et al 2008), spleen damage in common carp (Wang et al 2011), and phagocytic activity in Nile tilapia (Girón-Pérez et al 2006).

Chlorpyrifos causes endocrine disruption in fish: it lowered serum cortisol, oestradiol and testosterone levels, without change to gonad somatic indices, in Nile tilapia (Oruc 2010). Studies on marine mussels *Mytilus galloprovincialis* showed complex interactions between chlorpyrifos and 17 β -oestradiol in the digestive gland (Canesi et al 2011). It is embryotoxic to the crustacean *Daphnia magna* at environmental concentrations: chronic exposure at 0.1 μ g/L caused abnormalities in 20% of embryos (Palma et al 2009). It is also a developmental neurotoxicant in fish: exposure during the first 5 days after fertilisation resulted in impaired learning in zebrafish and reduced dopamine levels that persisted into adulthood (Eddins et al 2010).

A 2004 study found that low levels of chlorpyrifos suppressed olfactory function in salmonids when they examined sub-lethal neurotoxicity by recording odour-evoked field potentials from the sensory epithelium and olfactory forebrain using two natural odorants (taurocholic acid or L-serine). Chlorpyrifos decreased the amplitudes of the epithelial and bulbar responses to both odorants in a concentration-dependent manner. Benchmark concentrations for a 20% loss of sensory function were 0.72 μ g/L for chlorpyrifos.

Summary:

Chlorpyrifos is very acutely and chronically toxic to aquatic organisms. It causes motor in-coordination, delayed maturation, growth and reproduction impairment, deformities, and depressed populations. It is genotoxic, immunotoxic, an endocrine disruptor, embryotoxic, teratogenic, and a developmental neurotoxicant.

5.3 Classification and labelling

The following table lists the label statements for chlorpyrifos under EU legislation, based on the Globally Harmonised System (GHS) of classification and labelling, according to Annex VI to Regulation (EC) no 1272/2008.¹⁰

GHS classification and labelling

Category	Hazard Phrase
Acute Tox 3	H301 – toxic if swallowed
Aquatic Acute Tox 1	H400 – very toxic to aquatic life
Aquatic Chronic Tox 1	H410 – very toxic to aquatic life with long lasting effects

¹⁰ <http://esis.jrc.ec.europa.eu/index.php?PGM=cla>.

5.4 Summary of adverse effects

Genotoxicity and mutagenicity: The data is varied, but a variety of recent studies indicate that chlorpyrifos is mutagenic or genotoxic in human, rat, mouse, Chinese hamster, toad, fish, fruitfly and plant cells.

Cancer: Laboratory studies have not indicated cancer, other than those showing the proliferation of human breast cancer cells. However, there are a considerable number of epidemiological studies indicating an association between exposure to chlorpyrifos and cancer, particularly lung and rectal cancer. Weaker associations have been found with non-Hodgkin's lymphoma, leukaemia, brain, prostate and breast cancer.

Immunotoxicity: There is evidence of immune toxicity, including effects on lymphocytes, thymocytes, T cells, tumour necrosis factor, and autoimmunity.

Endocrine disruption: Chlorpyrifos is an endocrine disruptor; it inhibits metabolism of testosterone and oestradiol, and testosterone synthesis. It is anti-androgenic and oestrogenic, causing breast cancer cells to grow. It reduces serum levels of cortisol and thyroid hormone T4, induces alterations in thyroid and adrenal glands and differentially affects levels of thyroid-stimulating hormones in men and women. It is a breast cancer risk through its endocrine actions. It also affects gonadotrophin-releasing hormone, causes sex-select effects on insulin and cholesterol levels and causes endocrine disruption in fish and frogs.

Reproductive toxicity: Teratogenic effects observed in rats include skeletal malformations, small hind and fore limbs, lack of spinal development, absence of thoracic vertebrae and cleft palate; in human defects of the brain, eyes, ears, palate, teeth, heart, feet, nipples, and genitalia have been associated with gestational exposure to chlorpyrifos. Reproductive effects in animals include decreased foetal weight and viability; increased foetal death and early resorption; decreased sperm motility and count; decline in viability and developmental competence of oocytes. In humans chlorpyrifos exposure is associated with decreased birth weight and birth length; DNA damage in sperm, and decreased sperm concentration and sperm motility. Chlorpyrifos has been found in a number of human reproductive tissues including cervical fluid, sperm fluid, cord blood, meconium, and breast milk.

Developmental neurotoxicity: Chlorpyrifos is a potent developmental neurotoxin at low levels of exposure, below those that trigger foetal cholinesterase inhibition. This is demonstrated in numerous laboratory studies and number of recent epidemiological studies. Exposures in utero and in early childhood can lead to behavioural anomalies in adolescence and adulthood. Epidemiological studies found delayed cognitive and psychomotor development, and reduced IQ.

Ecotoxicity: The US EPA (2009a) reassessment of chlorpyrifos identified concerns about acute and chronic risks to birds, mammals, terrestrial invertebrates, fish, and aquatic invertebrates. Chlorpyrifos is very acutely and chronically toxic to aquatic organisms. It causes ataxia, delayed maturation, growth and reproduction impairment, deformities, and depressed populations. It is genotoxic, immunotoxic, an endocrine disruptor, embryotoxic, teratogenic, and a developmental neurotoxicant.

There is sufficient evidence that chlorpyrifos meets the Stockholm Convention Annex D 1(e) criterion of adverse effects.

6. Summary of POP properties

Persistence

Studies show that chlorpyrifos meets the Annex D 1(b)(i) threshold for persistence in soil and sediment under some conditions:

- In a number of studies based on termiticides treatments, in which high application rates (700-1,000 mg/kg) are used, half-lives have exceeded the Annex D threshold of 180 days, the highest reported being 1,576 days.
- A half-life of 223 days was reported for freshwater sediment under anaerobic conditions.

No half-lives exceeding the threshold appear to have been reported for water: however, with the half-life in seawater of 49.4 days at 10°C, more than double its half life at 20°C (15.2 days), it is entirely feasible that the half-life in seawater under Arctic and Antarctic conditions would exceed the Annex D threshold of 60 days.

Like endosulfan, chlorpyrifos is less persistent under tropical conditions characterised by faster microbial degradation, photodegradation and volatilisation, and more persistent under temperate conditions. Its persistence increases with decreased temperature, decreased pH, and decreased light. These are the conditions of the Arctic, and therefore it is reasonable to assume that persistence will be greater under Arctic conditions. In fact sampling has demonstrated its persistence in some Arctic conditions: it has been found in ice dating back to 1971. Calculated half-lives for Arctic conditions all exceed the Annex D thresholds.

There is sufficient evidence that chlorpyrifos also meets the Stockholm Convention Annex D 1(b)(ii) criterion of evidence that the chemical is persistent.

Bioaccumulation

Regulatory processes have not generally required bioaccumulation studies for chlorpyrifos, hence there are few available studies. Nevertheless those that are available do show a significant degree of bioaccumulation in a number of species, with one review from the manufacturer reporting a value of 5,100 for the bioaccumulation factor in fish, thus exceeding the threshold value of 5,000. Additionally, most reported values of log K_{ow} meet or exceed the threshold value of 5, with even the lowest value (4.7) being higher than that of the already listed POP lindane (3.5). Chlorpyrifos has been measured in fish in the Arctic.

There is sufficient evidence that chlorpyrifos meets the Stockholm Convention Annex D 1(c) criterion of evidence that the chemical is bioaccumulative.

Long-range transport

The atmospheric half-life, based on temperate conditions does not meet the annex D 1(d) threshold. However the lack of ultraviolet radiation and atmospheric moisture characteristic of the Arctic may account for the observed long-range transport of chlorpyrifos despite its generally assumed short atmospheric half-life.

Chlorpyrifos has been measured consistently in the Arctic, in ice, snow, fog, air, seawater, lake sediment, fish and vegetation, at significant levels such that some scientists have commented that it might have detrimental effects on the biota, especially aquatic organisms. It is amongst the pollutants with the highest concentrations present, sometimes more than endosulfan. Sampling of ice cores dates chlorpyrifos' appearance in the Arctic as at least as early as 1972 and it has been there ever since, at concentrations that are thought to have had the greatest impact of all pesticides in Svalbard, Norway. Input and burden of chlorpyrifos in the Arctic media is ongoing.

There is sufficient evidence that chlorpyrifos meets Stockholm Convention Annex D (1d) criterion of evidence that the chemical has the potential for long-range environmental transport.

Adverse effects

Genotoxicity and mutagenicity: The data is varied, but a variety of recent studies indicate that chlorpyrifos is mutagenic or genotoxic in human, rat, mouse, Chinese hamster, toad, fish, fruitfly and plant cells.

Cancer: laboratory studies have not indicated cancer, other than those showing the proliferation of human breast cancer cells. However, there are a considerable number of epidemiological studies indicating an association between exposure to chlorpyrifos and cancer, particularly lung and rectal cancer.

Immune toxicity: there is evidence of immune toxicity, including effects on lymphocytes, thymocytes, T cells, tumour necrosis factor, and autoimmunity.

Endocrine disruption: Chlorpyrifos inhibits metabolism of testosterone and oestradiol, and testosterone synthesis. It is anti-androgenic and oestrogenic, causing breast cancer cells to grow, and is a breast cancer risk through its endocrine actions. Chlorpyrifos reduces serum levels of cortisol and

thyroid hormone T4, induces alterations in thyroid and adrenal glands and differentially affects levels of thyroid-stimulating hormones in men and women.

Reproductive toxicity: Teratogenic effects in animal studies include skeletal malformations, small hind and fore limbs, lack of spinal development, absence of thoracic vertebrae and cleft palate; in human defects of the brain, eyes, ears, palate, teeth, heart, feet, nipples, and genitalia have been associated with gestational exposure to chlorpyrifos. Other effects in animals include decreased foetal weight and viability; increased foetal death and early resorption; decreased sperm motility and count; decline in viability and developmental competence of oocytes. In humans chlorpyrifos exposure is associated with decreased birth weight and birth length; DNA damage in sperm, and decreased sperm concentration and sperm motility. Chlorpyrifos has been found in a number of human reproductive tissues including cervical fluid, sperm fluid, cord blood, meconium, and breast milk.

Developmental toxicity: Chlorpyrifos is a potent developmental neurotoxin at low levels of exposure, below those that trigger foetal cholinesterase inhibition. This is demonstrated in numerous laboratory studies and a number of recent epidemiological studies. Exposures *in utero* and in early childhood can lead to behavioural anomalies in adolescence and adulthood. Epidemiological studies found delayed cognitive and psychomotor development, and reduced IQ.

Aquatic toxicity: Chlorpyrifos is very acutely and chronically toxic to aquatic organisms. It causes motor in-coordination, delayed maturation, growth and reproduction impairment, deformities, and depressed populations. It is genotoxic, immunotoxic, an endocrine disruptor, embryotoxic, teratogenic, and a developmental neurotoxicant.

There is sufficient evidence that chlorpyrifos meets the Stockholm Convention Annex D 1(e) criterion of evidence that the chemical causes adverse effects.

References

- Alavanja MCR, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, Knott C, Thomas K, Hoppin JA, Barker J, Coble J, Sandler DA, Blair A. 2003. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol* 157(9):800-14.
- Aldridge JE, Seidler FJ, Meyer A, Thillai I, Slotkin TA. 2003. Serotonergic systems targeted by developmental exposure to chlorpyrifos: effects during different critical periods. *Environ Health Perspect* 111(14):1736-43.
- Aldridge JE, Levin ED, Seidler FJ, Slotkin TA. 2005. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. *Environ Health Perspect* 113(5):527-31.
- Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B. 2008. Genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell electrophoresis. *Chemosphere* 7:1823-31.
- Amer SM, Farah OR. 1983. Cytological effects of pesticides. XII. Effects of the phosphorothioate insecticide dursban on the mitosis of *Vicia faba*. *Cytologia* 48:27-33.
- Amer SM, Aly FAE. 1992. Cytogenic effects of pesticides. IV. Cytogenic effects of the insecticides Gardona and Dursban. *Mutat Res* 279:165-70.
- Andersen HR, Vinggaard AM, Rasmussen TH, Gjermansen IM, Bonefeld-Jorgensen EC. 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity *in vitro*. *Toxicol Appl Pharmacol* 179:1-12.
- Ashauer R, Boxall A, Brown C. 2006. Uptake and elimination of chlorpyrifos and pentachlorophenol into the freshwater amphipod *Gammarus pulex*. *Arch Environ Contam Toxicol* 51:542-8.
- Baker PB, Bellamy DE. 2006. Field and laboratory evaluation of persistence and bioavailability of termiticides to desert subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). *J Econ Entomol* 99(4):1345-53.
- Barber D, Hunt J, Ehrlich M. 2001. Inhibition of calcium-stimulated ATPase in the hen brain P2 synaptosomal fraction by organophosphorus esters: relevance to delayed neuropathy. *J Toxicol Environ Health* 63:101-13.
- Baskaran S, Kookana RS, Naidu R. 1999. Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. *Pestic Sci* 55:1222-8.

- Bejarano A, Widenfalk A, Decho AW, Chandler GT. 2003. Bioavailability of the organophosphate insecticide chlorpyrifos to the suspension-feeding bivalve, *Mercenaria mercenaria*, following exposure to dissolved and particulate matter. *Environ Toxicol Chem* 22(9):2100-5.
- Bernabò I, Gallo L, Sperone E, Triperi S, Brunelli E. 2011. Survival, development, and gonadal differentiation in Raba dalmatina chronically exposed to chlorpyrifos. *J Exp Zool A Ecol Genet Physiol* 315:324-27.
- Blakely BR, Yole MJ, Brousseau P, Boermans H, Fournier M. 1999. Effect of chlorpyrifos on immune function in rats. *Vet Hum Toxicol* 41(3):140-4.
- Bondarenko S, Gan J, Haver DL, Kabashima JN. 2004. Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. *Environ Toxicol Chem* 23(11):2649-54.
- Bondarenko S, Gan J. 2004. Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environ Toxicol Chem* 23(8):1809-14.
- Budd R, O'green A, Goh KS, Bondarenko S, Gan J. 2011. Removal mechanisms and fate of insecticides in constructed wetlands. *Chemosphere* 83:1581-7.
- Cal EPA. 2008. Evidence on the developmental and reproductive toxicity of chlorpyrifos. Draft. California Environmental Protection Agency. http://oehha.ca.gov/prop65/hazard_ident/pdf_zip/ChlorpyrifosHID0908.pdf.
- Campbell CG, Seidler FJ, Slotkin TA. 1997. Chlorpyrifos interferes with cell development in rat brain regions. *Brain Res Bull* 43(2):179-89.
- Canesi L, Negri A, Barmo C, Banni M, Gallo G, Viarengo A, Dondero F. 2011. The organophosphate chlorpyrifos interferes with the responses of 17 β -estradiol in the digestive gland of marine mussel *Mytilus galloprovincialis*. *PLoS ONE* 6(5):e19803.
- Chai L-K, Mohd-Tahir N, Hansen HCB. 2008. Dissipation of acephate, chlorpyrifos, cypermethrin and their metabolites in a humid-tropical vegetable production system. *Pest Manag Sci* 65:189096.
- Chernyak SM, Rice CP, McConnell LL. 1996. Evidence of currently-used pesticides in air, ice, fog, seawater and surface microlayer in the Bering and Chukchi seas. *Mar Pollut Bull* 32(5):410-41.
- Colborn T. 2006. A case for revisiting the safety of pesticides: a closer look at neurodevelopment. *Environ Health Perspect* 114:10-17.
- Crumpton TL, Seidler FJ, Slotkin TA. 2000. Developmental neurotoxicity of chlorpyrifos in vivo and in vitro: effects on nuclear transcription factor involved in cell replication and differentiation. *Brain Res* 857:87-98.
- Cui Y, Guo J, Chen Z. 2011. Genotoxicity of chlorpyrifos and cypermethrin in ICR mouse lymphocytes. *Toxicol Mech Meth* 21(1):70-4.
- Dam K, Seidler FJ, Slotkin TA. 1998. Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration. *Dev Brain Res* 108:39-45.
- Dam K, Garcia SJ, Seidler FJ, Slotkin TA. 1999. Neonatal chlorpyrifos exposure alters synaptic development and neuronal activity in cholinergic and catecholaminergic pathways. *Brain Res Dev Brain Res* 116(1):9-20.
- Dam K, Seidler FJ, Slotkin TA. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. *Brain Res Dev Brain Res* 121(2):179-87.
- Das KP, Barone S Jr. 1999. Neuronal differentiation in PC12 cells is inhibited by chlorpyrifos and its metabolites: is acetylcholinesterase inhibition the site of action? *Toxicol Appl Pharmacol* 160(3):217-30.
- De Angelis S, Tassinari R, Maranghi F, Eusepi A, Di Virgilio A, Chiarotti F, Ricceri L, Pesciolini AV, Gilardi E, Moracci G, Calamandrei G, Olivieri A, Mantovani A. 2009. Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in Cd1 mice. *Toxicol Sci* 108(2):311-9.
- Dimitrov B, Gadeva P. 1997. Genotoxicity studies on the insecticide dursban in root meristem cells of *Crepis capillaris* L. *Environ Exper Bot* 37:199-209.
- Eder KJ, Clifford MA, Hedrick RP, Kohler H-R, Werner I. 2008. Expression of immune-regulatory genes in juvenile Chinook salmon following exposure to pesticides and infectious hematopoietic necrosis virus (IHNV). *Fish Shellfish Immunol* 25:508-16.
- Eddins D, Cerutti D, Williams P, Limney E, Levin ED. 2010. Zebrafish provide a sensitive model of persisting neurobehavioural effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. *Neurotox Teratol* 32(1):99-108.

- EFSA.2005. Review report for the active substance chlorpyrifos. SANCO/3059/99 - rev. 1.5, 3 June 2005. European Food Safety Authority. http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.selection.
- El-Amrani S, Pena-Abaurrea M, Sanz-Landaluze J, Ramos L, Guinea J, Camara C. 2012. Bioconcentration of pesticides in zebrafish eleutheroembryos (*Danio rerio*). *Sci Total Environ* 425:184-90.
- Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J, Samanic C, Sandler DP, Blair A, Alavanja MC. 2005. Pesticide use and breast cancer risk among farmers' wives in the Agricultural Health Study. *Am J Epidemiol* 161(2):121-35.
- Farag AT, El Okazy AM, El-Aswed AF. 2003. Developmental toxicity study of chlorpyrifos in rats. *Repro Toxicol* 17:203-8.
- Farag AT, Radwan AH, Sorour F, El Okazy A, El-Agamy E-S, El-Sebae AE-K. 2010. Chlorpyrifos induced reproductive toxicity in male mice. *Repro Toxicol* 29(1):80-5.
- Fortenberry GZ, Hu H, Turyk M, Barr DB, Meeker JD. 2012. Association between urinary 3,5,6-trichloro-2-pyridinol, a metabolite of chlorpyrifos and chlorpyrifos-methyl, and serum T4 and TSH in NHANES 1999-2002. *Sci Total Environ* 424:351-5.
- Flaskos J. 2012. The developmental neurotoxicity of organophosphorus insecticides: A direct role for the oxon metabolites. *Toxicol Letts* 209:86-93.
- Franke C, Studinger G, Berger G, Böhling S, Bruckmann U, Cohores-Fresenburg D, Jöhnke U. 1994. The assessment of bioaccumulation. *Chemosphere* 29(7):1501-14.
- Galloway T, Handy R. 2003. Immunotoxicity of organophosphate pesticides. *Ecotoxicology* 12:345-63.
- Garbarino JR, Snyder-Conn E, Lieker TJ, Hoffman GL. 2002. Contaminants in Arctic snow collected over Northwest Alaskan sea ice. *Water Air Soil Pollut* 139(1-4):183-214.
- Garcia SJ, Seidler FJ, Crumpton TL, Slotkin TA. 2001. Does the developmental neurotoxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells. *Brain Res* 891:54-68.
- Garcia SJ, Seidler FJ, Qiao D, Slotkin TA. 2002. Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. *Brain Res Dev Brain Res* 133(2):151-61.
- Gebremariam SY, Beutel MW, Yonge DR, Flury M, Harsh JB. 2012. Adsorption and desorption of chlorpyrifos to soils and sediments. *Rev Environ Contam Toxicol* 215:123-75.
- Ghisari M, Bonfeld-Jorgensen EC. 2006. Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. *Mol Cell Endocrin* 244:31-41.
- Girón-Pérez MI, Barcelos-García R, Vidal-Chavez ZG, Romero-Bañuelos CA, Robledo-Marengo ML. 2006. Effect of chlorpyrifos on the hematology and phagocytic activity of Nile tilapia cells (*Oreochromis niloticus*). *Toxicol Mech Methods* 16(9):495-9.
- Gore AC. 2001. Environmental toxicant effects on neuroendocrine function. *Endocrine* 14(2):235-46
- Gore AC. 2002. Organochlorine pesticides directly regulate gonadotrophin-releasing hormone expression and biosynthesis in the GT1-7 hypothalamic cell line. *Mol Cell Endocrin* 192:157-70.
- Gotoh M, Saito I, Huang J, Fukaya Y, Matsumoto T, Hosanaga N, Shibata E, Ichihara G, Kamujima M, Takeuchi Y. 2001. Changes in cholinesterase activity, nerve conduction velocity, and clinical signs and symptoms in termite control operators exposed to chlorpyrifos. *J Occup Health* 43:157-64.
- Gouin T. 2003. Appendix B: Comparison of methods for estimating degradation half-lives. In: *Long-Range Transport of Organic Contaminants: The Role of Air-Surface Exchange*. MSc thesis. Trent University, Peterborough, Ontario, Canada.
- Grigoryan H, Lockridge O. 2009. Nanoimages show disruption of tubulin polymerization by chlorpyrifos oxon: Implications for neurotoxicity. *Toxicol Appl Pharmacol* 240:143-8.
- Grünefeld HT, Bonfeld-Jorgensen EC. 2004. Effect of *in vitro* estrogenic pesticides on human oestrogen receptor alpha and beta mRNA levels. *Toxicol Letts* 151:467-80.
- Gupta SV, Mishra M, Sharma A, Balaji D, Kumar R, Mishra RK, Chowdhuri DK. 2010. Chlorpyrifos induces apoptosis and DNA damage in *Drosophila* through generation of reactive oxygen species. *Ecotox Environ Saf* 73:1415-23.

- Hageman KJ, Simonish SL, Campbell DH, Wilson GR, Landers DH. 2006. Atmospheric deposition of current-use and historic-use pesticides in snow in national parks in the western United States. *Environ Sci Technol* 40(10):3174-3180. Supporting Information.
- Hansen DJ, Goodman LR, Cripe GM, Macauley SF. 1986. Early life-stage toxicity test methods for gulf toadfish (*Opsanus beta*) and results using chlorpyrifos. *Ecotoxicol Environ Saf* 11:15-22.
- Harford AJ, O-Halloran K, Wright PFA. 2005. The effects of in vitro pesticide exposures on the phagocytic function of four native Australian freshwater fish. *Aquatic Toxicol* 75:330-42.
- Haviland JA, Butz DE, Porter WP. 2010. Long-term sex selective hormonal and behaviour alterations in mice exposed to low doses of chlorpyrifos in utero. *Repro Toxicol* 29(1):74-9.
- Hermanson MH, Isaksson E, Teixeira C, Muir DCG, Compher KM, Li YF, Igarashi M, Kamiyama K. 2005. Current-use and legacy pesticide history in the Austfonna Ice Cap, Svalbard, Norway. *Environ Sci Technol* 39(21):8163-9.
- Hodgson E, Rose RL. 2008. Metabolic interactions of agrochemicals in humans. *Pest Manag Sci* 64:617-21.
- Hoferkamp L, Hermanson MH, Muir DG. 2010. Current use pesticides in Arctic media; 2000–2007. *Sci Total Environ* 408(15):2985-94.
- Horton MK, Kahn LG, Perera F, Barr DB, Rauh V. 2012. Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? *Neurotoxicol Teratol* [Epub 20 Jul].
- Howard AS, Bucelli R, Jett DA, Bruun D, Yang DR, Lein PJ. 2005. Chlorpyrifos exerts opposing effects on axonal and dendritic growth in primary neuronal cultures. *Toxicol Appl Pharmacol* 207(2):112-24.
- HSDB. Hazardous Substances Data Bank. 2010. TOXNET, Toxicology Data Network, United States National Library of Medicine. <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~CQIjad:1>. Accessed June 2012.
- Icenogle LM, Christopher NC, Blackwelder WP, Caldwell DP, Qiao D, Seidler FJ, Slotkin TA, Levin ED. 2004. Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation. *Neurotoxicol Teratol* 26(1):95-101.
- Jameson RR, Seidler FJ, Qiao D, Slotkin TA. 2006. Chlorpyrifos affects phenotypic outcomes in a model of mammalian neurodevelopment: critical stages targeting differentiation in PC12 cells. *Environ Health Perspect* 114(5):667-72.
- Jantunen LM, Wong F, Bidleman TF, Stern G. 2007. Occurrence and levels of current-use and legacy pesticides in air: Leg 1 of ArcticNet 2007. Arctic Net. Collingwood, ON. http://www.arcticnet.ulaval.ca/pdf/posters_2007/jantunen_et_al.pdf.
- Jarvinen AW, Nordling BR, Henry ME. 1983. Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetylcholinesterase inhibition. *Ecotoxicol Environ Saf* 7:423-34.
- Karunanayake CP, Spinelli JJ, McLaughlin JR. 2012. Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. *J Agromed* 17(1):30-9.
- Kaur P, Grover IS. 1985. Cytological effects of some organophosphorous pesticides. I. Mitotic effects. *Cytologia* 50:187-97.
- Kenaga EE. 1980. Correlation of bioconcentration factors of chemicals in aquatic and terrestrial organisms with their physical and chemical properties. *Environ Sci Technol* 14(5):553-6.
- Kojima H, Katsura E, Takeuchi S, Niyama K, Kobayashi K. 2004. Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect* 112:524-31.
- Laabs V, Wehrhan A, Pinto A, Dores E, Amelung W. 2007. Pesticide fate in tropical wetlands of Brazil: An aquatic microcosm study under semi-field conditions. *Chemosphere* 67:975-89.
- Lal S, Saxena DM, Lal R. 1987. Bioconcentration and metabolism of DDT, fenitrothion and chlorpyrifos by the blue-green algae *Anabaena* sp. and *Aulosira fertilissima*. *Environ Pollut* 46:187-96.
- Landers DH, Simonich SL, Jaffe DA, Geiser LH, Campbell DH, Schwindt AR, Schreck CB, Kent ML, Hafner WD, Taylor HE, Hageman KJ, Usenko S, Ackerman LK, Schrlau JE, Rose NL, Blett TF, Erway MM. 2008. The Fate, Transport, and Ecological Impacts of Airborne Contaminants in Western National Parks (USA). Western Airborne Contaminants Assessment Project Final Report: Vol 1. EPA/600/R-07/138. U.S. Environmental Protection Agency, Corvallis.

- Lee WJ, Blair A, Hoppin JA, Lubin JH, Rusiecki JA, Sandler DP, Dosemeci M, Alavanja MCR. 2004. Cancer incidence among pesticide applicators exposed to chlorpyrifos in the agricultural health study. *J Nat Cancer Instit* 96(23):1781-9.
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MCR. 2007. Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer* 121:339-46.
- Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. *Brain Res Dev Brain Res* 130(1):83-9.
- Li S, Liu Y, Ni Z, Song X, Liu X, Zhao L. 1993. Mutagenicity of 24 organophosphorous pesticides determined by 6 short-term tests. *Chinese J Pharmacol Toxicol* 7:73-7.
- Lovasi GS, Quinn J, Rauh VA, Perera FP, Andres HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. 2011. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. *Am J Public Health* 101(1):63-70.
- Marshall WK, Roberts JR. 1978. Ecotoxicology of chlorpyrifos. National Research Council of Canada, NRC Associate Committee on Scientific Criteria for Environmental Quality, Subcommittee on Pesticides and Related Compounds. Publ. No. 16079.
- Meeker JD, Ravi SR, Barr DB, Hauser R. 2007. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. *Repro Toxicol* 25(2):184-91.
- Monnet-Tschudi F, Zurich MG, Schilter B, Costa LG, Honegger P. 2000. Maturation-dependent effects of chlorpyrifos and parathion and their oxygen analogs on acetylcholinesterase and neuronal and glial markers in aggregating brain cell cultures. *Toxicol Appl Pharmacol* 165:175-83.
- Mugni H, Demetrio P, Paracampo A, Pardi M, Bulus G, Bonetto C. 2012. Toxicity persistence in runoff water and soil in experimental soybean plots following chlorpyrifos application. *Bull Environ Contam Toxicol* 89(1):208-12.
- Muir DC, Teixeira C, Wania F. 2004. Empirical and modeling evidence of regional atmospheric transport of current-use pesticides. *Environ Toxicol Chem* 23(10):2421-32.
- Muir DCG, Teixeira CA, Alaee M, Hermanson M. 2007. Persistent organohalogen and current use pesticides in remote lake waters, sediments, and ice caps. In: Castro-Jiménez J, Eisenreich SJ, Vices I. 2007. *Persistent Organic Pollutants (POPs) in the European Atmosphere: An Updated Overview*. Institute for Environment and Sustainability. European Commission, Directorate-General, Joint Research Centre. EUR 22876 EN. Pp88-95.
- Mulla MS, Norland RL, Westlake WE, Dell B, Amant JS. 1973. Aquatic midge larvicides, their efficacy and residues in water, soil, and fish in a warm-water lake. *Environ Entomol* 2(1):58-65.
- Mulrooney JE, Davis MK, Wagner TL, Ingram RL. 2006. Persistence and efficacy of termiticides used in reconstruction treatments to soil in Mississippi. *J Econ Entomol* 92(2):469-75.
- Muto MA, Lobelle F Jr, Bidanset JH, Wurpel JN. 1992. Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to DURSABAN. *Vet Hum Toxicol* 34(6):498-501.
- Nandi S, Gupta PSP, Roy SC, Selvaraju S, Ravindra JP. 2009. Chlorpyrifos and endosulfan affect buffalo oocyte maturation, fertilization, and embryo development in vitro directly and through cumulus cells. *Environ Toxicol* 26(1):57-67.
- Navarro HA, Basta PV, Seidler FJ, Slotkin TA. 2001. Neonatal chlorpyrifos administration elicits deficits in immune function in adulthood: a neural effect. *Brain Res Dev Brain Res* 130(2):249-52.
- NMFS. 2008. National Marine Fisheries Service Endangered Species Section 7 Consultation Biological Opinion. EPA Registration of Pesticides Containing Chlorpyrifos, Diazinon, and Malathion (November 18, 2008). National Marine Fisheries Service. http://www.nmfs.noaa.gov/pr/pdfs/pesticide_biop.pdf.
- NRA. 2000. The NRA Review of Chlorpyrifos. Volume 1. National Registration Authority for Agricultural and Veterinary Medicines, Canberra. http://www.apvma.gov.au/products/review/docs/chlorpyrifos_summary.pdf.
- Odenkirchen EW, Eisler R. 1988. Chlorpyrifos hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report No.13. http://www.pwrc.usgs.gov/infobase/eisler/chr_13_chlorpyrifos.pdf.
- Ojha A, Yaduvanshi SK, Pant SC, Lomash V, Srivasta N. 2011. Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. *Environ Toxicol* [Epub July 22].

- Oruc EO. 2010. Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. *Pestic Biochem Physiol* 96:160-6.
- Ostrea EM, Morales V, Ngoumgna E, Prescilla R, Tan E, Hernandez E, Ramirez GB, Cifra HL, Manlapaz ML. 2002. Prevalence of fetal exposure to environmental toxins as determined by meconium analysis. *Neurotoxicology* 23(3):39.
- Palma P, Palma VL, Fernandes RM, Bohn A, Soares AMVM, Barbosa IR. 2009. Embryo-toxic effects of environmental concentrations of chlorpyrifos on the crustacean *Daphnia magna*. *Ecotox Environ Saf* 72(6):1714-8.
- Patnaik KK, Tripathy NK. 1992. Farm-grade chlorpyrifos (Durmet) is genotoxic in somatic and germ-line cells of *Drosophila*. *Mutat Res* 279:15-20.
- Perera FP, Rauh V, Tsai W-Y, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu Y-H, Diaz D, Dietrich J, Whyatt RM. 2003. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environ Health Perspect* 111(2):201-5.
- Prakash A, Khan S, Aggarwal M, Telang AG, Malik JK. 2009. Chlorpyrifos induces apoptosis in murine thymocytes. *Toxicol Letts* S83:V60.
- Prasertsup P, Ariyakanon N. 2011. Removal of chlorpyrifos by water lettuce (*Pistia stratiotes* L.) and duckweed (*Lemna minor* L.). *Int J Phytoremediation* 13(4):383-95.
- PPDB. 2012. Pesticide Properties DataBase, University of Hertfordshire. <http://sitem.herts.ac.uk/aeru/footprint/en/>. Accessed July 2012.
- Qiao D, Seidler F, Padilla S, Slotkin TA. 2002. Developmental neurotoxicity of chlorpyrifos: What is the vulnerable period? *Environ Health Perspect* 110:1097-103.
- Qiao D, Seidler F, Tate CA, Cousins MM, Slotkin TA. 2003. Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers emerge postnatally and continue into adolescence and adulthood. *Environ Health Perspect* 111:536-44.
- Racke KD. 1993. Environmental fate of chlorpyrifos. *Rev Environ Contam Toxicol* 131:1-150.
- Racke KD, Fontaine DD, Yoder RN, Miller JR. 1994. Chlorpyrifos degradation in soil at termiticidal application rates. *Pestic Sci* 42:43-51.
- Racke KD, Steele KP, Yoder RN, Dick WA, Avidov E. 1996. Factors affecting the hydrolytic degradation of chlorpyrifos in soil. *J Agric Food Chem* 44:1582-92.
- Rahman MF, Mahboob M, Danadevi K, Saleha BS, Grover P. 2002. Assessment of genotoxic effects of chlorpyrifos and acephate by the comet assay in mice leucocytes. *Mutat Res* 516:139-47.
- Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics* 118:1845-59.
- Rauh VA, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, Whyatt R. 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect* 119(8):1196-201.
- Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS. 2012. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *PNAS* 109(20):7871-6.
- Rawlings NC, Cook SJ, Waldbillig D. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J Toxicol Environ Health Part A* 54:21-36.
- Rice CP, Chernyak SM. 1997. Marine arctic fog: An accumulator of currently used pesticides. *Chemosphere* 35(4):867-78.
- Rorjje E, Verbruggen EMJ, Holander A, Traas TP, Jansen MPM. 2011. Identifying potential POP and PBT substances. Development of a new Persistence/Bioaccumulation-score. RIVM Report 601356001/2011. National Institute for Public Health and the Environment. <http://www.rivm.nl/bibliotheek/rapporten/601356001.pdf>.
- Rowsey PJ, Gordon CJ. 1999. Tumor necrosis factor is involved in chlorpyrifos-induced changes in core temperature in the female rat. *Toxicol Letts* 109:51-9.
- Ruggirello RM, Hermanson MH, Isaksson E, Teixeira C, Forsstrom S, Muir DCG, Pohjola V, van de Wal R, Meijer HAJ. 2010. Current use and legacy pesticide deposition to ice caps on Svalbard, Norway. *J Geophys Res* 115. doi:10.1029/2010JD014005.

- Samarawickrema N, Pathmeswaran A, Wickremasinghe R, Peiris-John R, Karunaratna M, Buckley N, Dawson A, de Silva J. 2008. Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. *Clin Toxicol* 46:489-95.
- Sandahl JF, Baldwin DH, Jenkins J, Scholz NL. 2004. Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences*. 61(3):004-11.
- Sandal S, Yilmaz B. 2011. Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. *Environ Toxicol* 26(5):433-42.
- Sardar D, Kole RK. 2005. Metabolism of chlorpyrifos in relation to its effect on the availability of some plant nutrients. *Chemosphere* 61:1273-80.
- Schuh RA, Lein PJ, Beckles RA, Jett DA. 2002. Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca²⁺/cAMP response element binding protein in cultured neurons. *Toxicol Appl Pharmacol* 182:176-85.
- Serrano R, Lopez FJ, Hernandez F, Pena JB. 1997. Bioconcentration of chlorpyrifos, chlorfenvinphos, and methidathion in *Mytilus galloprovincialis*. *Bull Environ Contam Toxicol* 59:968-75.
- Sherman JD. 1996. Chlorpyrifos (Dursban)-associated birth defects: report of four cases. *Arch Environ Health* 51(1):5-8.
- Slotkin TA. 1999. Developmental cholinotoxicants: nicotine and chlorpyrifos. *Environ Health Perspect* 107(suppl 1):71-80.
- Slotkin TA. 2004. Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. *Neurotoxicology* 25:631-40.
- Slotkin TA, Cousins MM, Tate CA, Seidler FJ. 2001. Persistent cholinergic presynaptic deficits after neonatal chlorpyrifos exposure. *Brain Res* 902(2):229-43.
- Slotkin TA, Brown KK, Seidler FJ. 2005. Developmental exposure of rats to chlorpyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. *Environ Health Perspect* 113(10):1291-4.
- Slotkin TA, Levin ED, Seidler FJ. 2006. Comparative developmental neurotoxicity of organophosphate insecticides: effects on brain development are separable from systemic toxicity. *Environ Health Perspect* 114:746-51.
- Slotkin TA, Seidler FJ, Fumagalli F. 2007. Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. *Environ Health Perspect* 115:909-16.
- Sobti RC, Krishan A, Pfaffenberger CD. 1982. Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. *Mutat Res* 102:89-102.
- Song X, Lin F, Jiang Y, Liu X, Zhang P, Liu Y. 1997. Mutagenicity of 19 organophosphorus pesticides in *Saccharomyces cerevisiae* D61.M. *Chinese J Pharmacol Toxicol* 11:291-3.
- Song X, Violin JD, Seidler FJ, Slotkin TA. 1998. Modeling the developmental neurotoxicity of chlorpyrifos in vitro: macromolecule synthesis in PC12 cells. *Toxicol Appl Pharmacol* 151(1):182-91.
- Stapleton AR, Chan VT. 2009. Subtoxic chlorpyrifos treatment resulted in differential expression of genes implicated in neurological functions and development. *Arch Toxicol* 83:319-33.
- Thomas CN, Mansingh A. 2002. Bioaccumulation, elimination, and tissue distribution of chlorpyrifos by red hybrid *Tilapia* in fresh and brackish waters. *Environ Technol* 23(11):1313-23.
- Thrasher JD, Madison R, Broughton A. 1993. Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. *Arch Environ Health* 48(2):89-93.
- Thrasher JD, Heuser G, Broughton A. 2002. Immunological abnormalities in humans chronically exposed to chlorpyrifos. *Arch Environ Health* 57(3):181-7.
- Tian Y, Yamauchi T. 2003. Micronucleus formation in 3-day mouse embryos associated with maternal exposure to chlorpyrifos during the early preimplantation period. *Repro Toxicol* 17:401-5.
- Tian Y, Ishikawa H, Yamaguchi T, Yamauchi T, Yokoyama K. 2005. Teratogenicity and developmental toxicity of chlorpyrifos maternal exposure during organogenesis in mice. *Repro Toxicol* 20:267-71.
- US EPA. 2006. Reregistration Eligibility Decision for Chlorpyrifos. United States Environmental Protection Agency, Washington, D.C. http://www.epa.gov/oppsrrd1/REDs/factsheets/chlorpyrifos_fs.htm.

- US EPA. 2009a. Chlorpyrifos Summary Document Registration Review: Initial Docket March 2009. Docket Number: EPA-HQ-OPP-2008-0850. Case #0100. United States Environmental Protection Agency, Washington, D.C.
- US EPA. 2009b. Memorandum. Chlorpyrifos. Revised Human Health Assessment Scoping Document in Support of Registration Review. February 9. From Drew D, Britton W, Doherty J, Risk Assessment Branch V, Health Effects Division to Santora K, Myers M, Reregistration Branch 2, Special Review and Reregistration Division (7508P). Office of Pesticide Programs, United States Environmental Protection Agency, Washington, D.C.
- Usmani KA, Rose RL, Hodgson E. 2003. Inhibition and activation of the human liver and human cytochrome P450 3A4 metabolism of testosterone by deployment-related chemicals. *Drug Metabol Disp* 31:384-91.
- Usmani KA, Cho TM, Rose RL, Hodgson E. 2006. Inhibition of the human liver microsomal and human cytochrome P450 1A2 and 3A4 metabolism of estradiol by deployment-related and other chemicals. *Drug Metabol Disp* 34:1606-14.
- Veith GD, DeFoe DL, Bergstedt BV. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. *J Fish Res Board Can* 36(9):1040-8.
- Ventura C, Núñez M, Miret N, Lamas DM, Randi A, Venturino A, Rivera E, Cocca C. 2012. Differential mechanisms of action are involved in chlorpyrifos effects in estrogen-dependent or -independent breast cancer cells exposed to low or high concentrations of the pesticide. *Toxicol Letts* 213:184-93.
- Viswanath G, Chatterjee S, Dabral S, Nanguneri SR, Divya G, Roy P. 2010. Anti-androgenic endocrine disrupting activities of chlorpyrifos and piperophos. *J Steroid Biochem Mol Biol* 120:22-9.
- Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP, Blair A. 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Canc Causes Contr* 12:509-17.
- Wang X, Xing H, Li X, Xu S, Wang X. 2011. Effects of atrazine and chlorpyrifos on the mRNA levels of IL-1 and IFN- γ in immune organs of common carp. *Fish Shellfish Immunol* 31(1):126-33.
- Welling W, de Vries JW. 1992. Bioconcentration kinetics of the organophosphorus insecticide chlorpyrifos in guppies (*Poecilia reticulata*). *Ecotoxicol Environ Saf* 23:64-75.
- Whitney KD, Seidler FJ, Slotkin TA. 1995. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. *Toxicol Appl Pharmacol* 134:53-62.
- WHO. 2009. WHO Specifications and Evaluations for Public Health Pesticides. Chlorpyrifos *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. World Health Organization, Geneva. http://www.who.int/whopes/quality/Chlorpyrifos_WHO_specs_eval_Mar_2009.pdf.
- Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP. 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect* 112(10):1125-32.
- Woodburn KB, Hansen SC, Roth GA, Strauss K. 2003. The bioconcentration and metabolism of chlorpyrifos by the eastern oyster, *Crassostrea virginica*. *Environ Toxicol Chem* 22(2):276-84.
- Woodruff RC, Phillips JP, Irwin D. 1983. Pesticide-induced complete and partial chromosome loss in screens with repair-defective females of *Drosophila melanogaster*. *Environ Mutagen* 5(6):835-6.
- Xing H, Li S, Wang Z, Gao X, Xu S, Wang X. 2012. Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. *Chemosphere* 88:377-83.
- Yang X-B, Ying G-G, Peng P-A, Wang L, Zhao J-L, Zhang L-J, Yuan P, He H-P. 2010. Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil. *J Agric Food Chem* 58:7915-21.
- Yin XH, Zhu GN, Li XB, Liu SY. 2009. Genotoxicity evaluation of chlorpyrifos to amphibian Chinese toad (Amphibian: Anura) by comet assay and micronucleus test. *Mutat Res/Genetic Toxicol Environ Mutagen* 680(1-2):2-6.
- Yoshida S, Taguchi S, Hori S. 2004. Chlorpyrifos and S-421 residues in indoor air and polished rice around nine years after application for termite control. *J Soc Indoor Environ Japan* 7:7-15.
- Zhong G, Xie Z, Cai M, Moller A, Sturm R, Tang J, Zhang G, He J, Ebinghaus R. 2012. Distribution and air-sea exchange of current-use pesticides (CUPs) from East Asia to the high Arctic Ocean. *Environ Sci Technol* 46:259-67.