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Persistent Organic Pollutants Review Committee
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Item 4 (a) (ii) of the provisional agenda**

Technical work: consideration of draft risk profiles: methoxychlor

Draft risk profile: Methoxychlor

Note by the Secretariat

I. Introduction

1. At its fifteenth meeting, the Persistent Organic Pollutants Review Committee adopted decision POPRC-15/3 on methoxychlor (UNEP/POPS/POPRC.15/7, annex I), by which the Committee decided to establish an intersessional working group to further review the proposal to list the chemical in Annexes A, B and/or C to the Convention (UNEP/POPS/POPRC.15/4) and to prepare a draft risk profile in accordance with Annex E to the Convention.

2. In accordance with decision POPRC-15/3 and the workplan adopted by the Committee (UNEP/POPS/POPRC.15/7, annex III), the intersessional working group has prepared a draft risk profile, which is set out in the annex to the present note, without formal editing. A compilation of comments and responses relating to the draft risk profile is set out in document UNEP/POPS/POPRC.16/INF/5.

II. Proposed action

3. The Committee may wish:

(a) To adopt, with any amendments, the draft risk profile set out in the annex to the present note;

(b) To decide, in accordance with paragraph 7 of Article 8 of the Convention and on the basis of the risk profile, whether methoxychlor is likely, as a result of its long-range environmental transport, to lead to significant adverse human health and/or environmental effects, such that global action is warranted;

(c) To agree, depending on the decision taken under subparagraph (b) above:

(i) To invite all Parties and observers to provide information pursuant to Annex F to the Convention, to establish an intersessional working group to develop a draft risk management evaluation and to agree on a workplan for completing that draft evaluation; or

(ii) To make the risk profile available to all Parties and observers and set the proposal aside.

* Reissued for technical reasons on 2 December 2020.

** UNEP/POPS/POPRC.16/1.

Annex

Methoxychlor

Draft risk profile

Prepared by the intersessional working group of the
Persistent Organic Pollutants Review Committee

June 2020

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Executive summary

1. The POPs Review Committee at its fifteenth meeting concluded that methoxychlor fulfilled the screening criteria in Annex D (decision POPRC-15/3) and to prepare a risk profile in accordance with Annex E to the Convention.

2. Methoxychlor is an organochlorine pesticide (OCP) which has been used as a replacement for DDT. It has been restricted/banned in several countries for more than 15 years. In response to the call for information (Annex E information submission (2019)), no Parties have indicated current use of methoxychlor. However, a literature search made for the purpose of drafting the risk profile suggests that recent use of methoxychlor may have occurred in certain countries. In 1975, three U.S. companies produced 2500 tonnes of methoxychlor. The production decreased to 193 tonnes in 1991. After 1992, production of methoxychlor in the U.S. was significantly reduced until its ban in 2000. No information on the current production or use of methoxychlor at a global scale is publicly available. Methoxychlor does not occur naturally in the environment. It is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Smaller amounts of methoxychlor may be released to the environment during its production, formulation, storage, shipment and disposal. Based on a maximum historical estimate of 8,000 tonnes/year of methoxychlor produced worldwide (circa 1975), peak historical atmospheric releases during production were estimated to be up to 4 tonnes/year. In the U.S., a total of 1.04 tonnes of methoxychlor was released in 2018 from on- and off-site disposal (or other releases) (US EPA, 2020a).

3. Methoxychlor is persistent in the environment. Numerous studies have reported detections and/or quantifications of methoxychlor in sediment, water, seawater, groundwater, drinking water and in a variety of biota. Contamination with methoxychlor is especially apparent in the vicinity of intensive agricultural activities as a result of the historical use of methoxychlor as a pesticide. Based on a weight-of-evidence approach, the results from the laboratory study and monitoring data indicate that methoxychlor is persistent in aerobic sediments and may be persistent in some anaerobic sediments. Measured data have indicated that methoxychlor continued to be detected in surface waterbodies in Europe and Canada, and in French groundwaters, years after it was phased out, thus providing some evidence that the substance is persistent in water. Monitoring data from an Arctic lake and surface seawater in a region covering the North Pacific to the Arctic Ocean, further indicate that the substance may be persistent in the surface water and marine water compartments. Based on a weight-of-evidence approach, the results from the laboratory studies and monitoring data indicate that methoxychlor may be persistent in some aerobic soils. However, the presence of methoxychlor in the surface water, seawater and soil mentioned above can also be a result of long-range transport.

4. Methoxychlor is a strongly hydrophobic substance with an experimental octanol-water partition coefficient (log K_{ow} value) of 5.08. The BCF values for methoxychlor vary largely between different aquatic species as a result of species differences in the capacity to metabolise and excrete methoxychlor (BCFs in the range of 667–8,300). Laboratory studies indicate that methoxychlor has a bioaccumulation potential in some fish species with BCF values > 5,000. Supporting information in a bivalve (BCF of 12,000) and in snails (BCF in the range of 5,000–8,570) also indicate a bioaccumulation potential in aquatic invertebrates. Combination of the bioaccumulation potential of methoxychlor with a high toxicity and high ecotoxicity gives reason for concern. Toxicokinetic and metabolism studies would suggest that methoxychlor does not accumulate in mammals.

5. Based on measured data, methoxychlor has been frequently detected in the environment including in urban cities, rural areas and in regions that used methoxychlor as a pesticide in agricultural activities. It has been found in the Arctic (in air, snow, ice cores, lake waters and seawater, in biota samples (terrestrial, avian and marine)) and Antarctic (in marine biota samples) regions far away from any sources of release thus suggesting that long-range atmospheric and oceanic transport has occurred.

6. Based on measured data, exposure of the general population takes place by consumption of contaminated food and drinking water, and by respiratory uptake of dust and aerosols containing methoxychlor and through oral uptake of dust and soil. Small children may play close to the ground and are therefore more likely than adults to come in contact with dirt and dust. Children may also intentionally or unintentionally ingest dust or soil that contains low levels of methoxychlor. Methoxychlor has been detected in human serum, adipose tissues, umbilical cord blood and human breast milk. Based on measured data, children can be exposed to methoxychlor *in utero* via the placenta and after birth via lactation.

7. Due to its persistence, methoxychlor is still found in drinking water, waterbodies and sediments, in regions where regulations and phase-outs have been implemented. Environmental trend data are not available, and the available measured data are insufficient to draw a clear conclusion on trends. However, from 1999 to 2014, it was observed that the concentrations of methoxychlor in Southern elephant seals in Antarctica had increased. No data are available on exposure trends of methoxychlor in humans.

8. Concerns exist regarding methoxychlor's toxicity to aquatic organisms, wildlife and human health. Methoxychlor is very toxic to aquatic invertebrates and fish. Methoxychlor is suspected to have endocrine disruptive effects in fish, amphibian, and sea urchin fertility, growth, and development. It also has a potential endocrine

disrupting property with toxic effects to reproduction in mammals in both female and male rats. Furthermore, observations in rats indicate that methoxychlor has the potential to promote the epigenetic transgenerational inheritance of disease and associated sperm epimutations. Methoxychlor has been demonstrated to be a neurotoxicant at high doses and can act on cognitive functions on the developing brain at lower doses. In addition, developmental and adult dietary exposure to methoxychlor in rats modulates immune responses. Finally, in certain defined cases, the simultaneous exposure of methoxychlor to other environmental chemicals has resulted in additive effects.

9. Methoxychlor has been detected in environmental compartments such as surface water, seawater, groundwater, drinking water, sediment, atmosphere, biota (including wildlife) and humans globally. Methoxychlor is persistent, bioaccumulative, toxic to aquatic organisms and to terrestrial animals (including humans) and transported to locations far from its production and use. Therefore, it is concluded that methoxychlor is likely to lead to adverse human health and/or environmental effects such that global action is warranted.

1. Introduction

10. In May 2019, the European Union and its Member States submitted a proposal to list methoxychlor in Annex A to the Stockholm Convention. The proposal (UNEP/POPS/POPRC.15/4) was submitted in accordance with Article 8 of the Convention and it was reviewed by the Persistent Organic Pollutants Review Committee (POPRC) at its fifteenth meeting held in October 2019.

1.1 Chemical identity of the proposed substance

11. Pure methoxychlor is a pale-yellow powder that has a slightly fruity or musty odour (Agency for Toxic Substances and Disease Registry (ATSDR), 2002).

Table 1: Names and registry numbers

Common name	Methoxychlor*
IUPAC	1,1'-(2,2,2-trichloroethane-1,1-diyl)bis(4-methoxybenzene) 1-methoxy-2-[2,2,2-trichloro-1-(4-methoxyphenyl)ethyl]benzene 1,1'-(2,2,2-trichloroethane-1,1-diyl)bis(2-methoxybenzene)
CAS registry number (non-exhaustive list)	72-43-5; 30667-99-3; 76733-77-2; 255065-25-9; 255065-26-0; 59424-81-6; 1348358-72-4
EC number	200-779-9
Synonyms and Trade name	1,1-Bis(*para*-methoxyphenyl)-2,2,2-trichloroethane 2,2-Bis(*para*-methoxyphenyl)-1,1,1-trichloroethane 2,2-Di-*para*-anisyl-1,1,1-trichloroethane *para*,*para'*-Dimethoxydiphenyltrichloroethane Dimethoxy-DDT Dimethoxy-DT Di(*para*-methoxyphenyl)trichloromethyl methane DMDT *para*,*para'*-DMDT ENT1716 Higalmetox Methoxychlore Maralate Marlate OMS 466 *para*,*para'*-Methoxychlor Metox Methoxy-DDT Prentox 1,1,1-Trichloro-2,2-bis(*para*-methoxyphenyl)ethane 1,1,1-Trichloro-2,2-di(4-methoxyphenyl)ethane 1,10-(2,2,2-Trichloroethylidene)bis(4-methoxy-benzene) Ethane, 1,1,1-trichloro-2-(*o*-methoxyphenyl)-2-(*p*-methoxyphenyl)- 2,4'-Methoxychlor *o*,*p*-Methoxychlor *o*,*p'*-Methoxychlor Benzene, 1,1'-(2,2,2-trichloroethylidene)bis[2-methoxy- Benzene, 1-methoxy-3-[2,2,2-trichloro-1-(4-methoxyphenyl)ethyl]- Benzene, 1,1'-(2,2,2-trichloroethylidene)bis[3-methoxy-
Abbreviations	MXC

*Methoxychlor refers to any possible isomer of dimethoxydiphenyltrichloroethane or any combination thereof.

Table 2: Structure

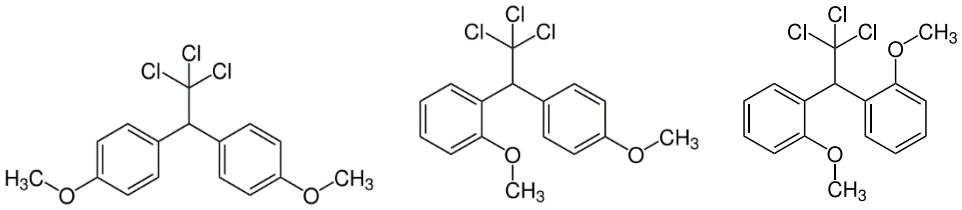
Molecular formula	$C_{16}H_{15}Cl_3O_2$
Molecular mass	345.65 g/mol
Structural formulas examples	

Table 3: Overview of relevant physicochemical properties of methoxychlor

Property	Value	References
Physical state at 20°C and 101.3 kPa	Solid (pale-yellow powder)	ATSDR, 2002
Melting/freezing point (MP)	87°C *(experimental)* 129.34°C *(EPI Suite, MPBPVP v1.43 estimate, Mean or weighted MP) (within applicability domain (parametric))*	Lide, 2007 US EPA, 2012
Boiling point (BP)	346°C *(experimental)* 377.87°C *(EPI Suite, MPBPVP v1.43 estimate, Adapted Stein & Brown method) (within applicability domain (parametric))*	US EPA, 2012 US EPA, 2012
Vapour pressure	5.56 x 10^{-3} Pa at 25°C *(EPI Suite, MPBPVP v1.43 estimate, modified grain method; input experimental MP and BP, temp 25°C) (within applicability domain)*	US EPA, 2012
Henry's Law constant	2.03 x 10^{-7} atm.m^3/mol at 25°C (or 2.06 x 10^{-2} Pa.m^3/mol) *(experimental) (in HENRYWIN validation set and calculated from Altschuh et al. (1999) study)* 9.75 x 10^{-8} atm.m^3/mol (or 9.88 x 10^{-3} Pa.m^3/mol) (*EPI Suite HENRYWIN v3.20 estimate, Bond method)(within applicability domain)*	Altschuh *et al*., 1999 US EPA, 2012
Water solubility	0.040 mg/L at 24°C *(experimental, 99% purity)* 0.10 mg/L at 25–45°C *(experimental, shake flask-UV)* 0.12 mg/L at 25°C *(experimental, in WSKOW training set)* 0.302 mg/L at 25°C *(EPI Suite, WSKOW v1.42 estimate) (within applicability domain)*	Verschueren, 1996 Richardson and Miller, 1960 Zepp *et al.*, 1976 US EPA, 2012
Organic carbon normalized adsorption coefficient (log K_{oc})	4.9 *(experimental, in KOCWIN training set)* 4.43 *(EPI Suite, KOCWIN v2.00 estimate, MCI method) (within applicability domain)*	Schüürmann *et al*., 2006 US EPA, 2012
Octanol/water partition coefficient (log K_{ow})	5.08 *(experimental, in KOWWIN training set)* 5.67 *(EPI Suite, KOWWIN v1.68 estimate) (within applicability domain)*	Karickhoff *et al*., 1979 US EPA, 2012
Octanol/air partition coefficient (log K_{oa})	10.48 (*experimental, GC retention time method*) 10.161 *(EPI Suite, KOAWIN v1.10 estimate; log Kow and Henry's Law constant experimental values as input) (within applicability domain (parametric))*	Odabasi and Cetin, 2012 US EPA, 2012
Air/water partition coefficient (log K_{aw})	-5.081 *(EPI Suite, KOAWIN v1.10, calculated from experimental Henry's Law Constant)*	US EPA, 2012

1.2 Conclusion of the Review Committee regarding Annex D information

12. The Persistent Organic Pollutants (POPs) Review Committee evaluated the proposal by the European Union to list methoxychlor under the Stockholm Convention at its fifteenth meeting. The Committee concluded that methoxychlor met the screening criteria specified in Annex D (decision POPRC-15/3).

1.3 Data sources

13. The draft risk profile is based on the following data sources:

(a) Proposal submitted by the European Union and its Member States that are Parties to the Convention (UNEP/POPS/POPRC.15/4), 2019;

(b) Information submitted by Parties and observers according to Annex E of the Convention (Annex E, 2019 and 2020): Austria, Canada, Egypt, Hungary, Monaco, New Zealand, Qatar, Republic of Belarus, Republic of Korea, Romania, State of Palestine, Thailand, the Netherlands, International Pollutants Elimination Network (IPEN) and Alaska Community Action on Toxics (ACAT);

(c) Peer-reviewed scientific journals, as well as information from reports such as: Toxicological Profile for Methoxychlor (ATSDR, 2002 and 2012); Methoxychlor in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality (WHO, 2004); and the Protection of the Marine Environment of the North-East Atlantic (OSPAR) Background Document on Methoxychlor (OSPAR, 2004).

1.4 Status of the chemical under International Conventions

14. Methoxychlor is subject to a limited number of international actions and Conventions, as follows:

(a) The OSPAR Commission included methoxychlor in the List of Chemicals for Priority Action in 2000 (OSPAR, 2004);

(b) Serbia as part of the UNECE Working Group on Environmental Monitoring and Assessment (WGEMA) added methoxychlor as a new parameter for measurement under diffuse soil contamination monitoring.[1] Methoxychlor was then added to the Shared Environmental Information System (SEIS) of the WGEMA;

(c) Methoxychlor has a water-quality objective of 0.04 ppb for the Great Lakes.[2] The water-quality objectives related to persistent organic substances have been established under the responsibility of the International Joint Commission according to the provisions of the 1978 Great Lakes Water Quality Agreement;

(d) According to the State of Palestine (Annex E, 2019), methoxychlor is subject to the terms and conditions of the Basel Convention in accordance with Annex I (category of wastes to be controlled) category Y3 (waste pharmaceuticals, drugs and medicines).

2. Summary information relevant to the risk profile

2.1 Sources

2.1.1 Production, trade, stockpiles

15. According to ATSDR (2002), methoxychlor was first synthesised in 1893 by the reaction of chloral hydrate with anisole in the presence of acetic acid and sulfuric acid. It is produced commercially by the condensation of anisole with chloral in the presence of an acidic condensing agent (the International Agency for Research on Cancer (IARC), 1979; Sittig, 1980 as cited in ATSDR, 2002). Commercial production of methoxychlor in the United States (U.S.) was first reported in 1946. In 1975, three U.S. companies produced methoxychlor (IARC, 1979 as cited in ATSDR, 2002). Very few data on the levels of production of methoxychlor are readily available. According to Götz *et al.* (2008), the peak production of methoxychlor in the U.S. was in the late 1970s to early 1980s. After that period, production of methoxychlor continuously decreased over time. U.S. production in 1975 was 2500 tonnes (IARC 1979, as referenced by ATSDR 2002), decreasing to 193 tonnes in 1991 (Kincaid Enterprises 1992, as referenced by ATSDR 2002). After 1992, production of methoxychlor in the U.S. was significantly reduced until its ban in 2000 (US EPA, 2004).

16. Methoxychlor is not approved as a plant protection product (PPP) in the European Union (EU) (Commission Regulation (EC) No 2076/2002) and the authorisations for use as PPP were withdrawn by 25 July 2003. Some EU Member States had imposed a ban of the use prior to 2003. Based on the WWF (2001) report for OSPAR (as cited in OSPAR, 2004), no existing producers or importers of methoxychlor have been reported in Europe since it has been phased out in 2002. Methoxychlor is not approved as an active substance in the EU under the Commission Regulation (EC) No 2032/2003 concerning the market and use of biocidal products as of 1 September 2006. Methoxychlor is not

[1] This information is available on the UNECE website at the following link: https://www.unece.org/fileadmin/DAM/env/europe/monitoring/15thMeeting/Official/Questionnaire_2014_Eng_final_Serbia.pdf

[2] This information is available on the UNECE website at the following link: http://www.unece.org/fileadmin/DAM/env/water/publications/documents/Reco_E/Reco_waterquality_crit_obj_WS1.pdf

approved for use as a veterinary medicinal product in the EU according to Regulation (EC) No 726/2004 on the procedures for the authorisation and supervision of medicinal products for human and veterinary use.

17. Monaco, Qatar, Republic of Korea and State of Palestine do not currently produce methoxychlor (Annex E (2019) submission information). Canada and Thailand do not currently produce, import or export methoxychlor (Annex E, 2019). Furthermore, methoxychlor has never been manufactured in Belarus (Annex E, 2019). No stockpiles of methoxychlor have been registered in Costa Rica. Egypt does not currently produce or import methoxychlor (it has been banned in Egypt since 1996 by ministerial decree no. 55/1996 (Annex E, 2019)). Vanuatu does not currently import methoxychlor. There is no official data of import of methoxychlor in Ghana. Within the framework of the import authorization procedure, Mexico has not registered any import of methoxychlor since 2010.

18. No further data on the current levels of production or import of methoxychlor are publicly available.

2.1.2 Uses

19. Methoxychlor is an organochlorine pesticide (OCP) and it has been used as a replacement for DDT, a structural analogue. In veterinary practices, methoxychlor was used as an ectoparasiticide[3] (US EPA, 2000). Methoxychlor was also used against the elm bark-beetle vectors of Dutch elm disease (US EPA, 2000). Methoxychlor has been used as an insecticide against a wide range of pests, including houseflies and mosquitos, cockroaches, chiggers, and various arthropods commonly found on field crops, vegetables, fruits, stored grain, livestock, and domestic pets (EPA[4], 1988b and Verschueren, 1983 as cited in ATSDR, 2002). Methoxychlor can be applied to large areas such as beaches, estuaries, lakes, and marshes for control of fly and mosquito larvae by aerial application (EPA, 1988b as cited in ATSDR, 2002). Other uses include the spray treatment of barns, grain bins, mushroom houses, and other agricultural premises and the spraying or fogging of garbage containers, sewer manholes, and sewage disposal areas (EPA, 1988b as cited in ATSDR, 2002). In the U.S., approximately 28% of methoxychlor was used for home and garden purposes, 15% for industrial and commercial purposes, and 57% for agricultural purposes (Kincaid Enterprises, 1992 as cited in ATSDR, 2002). Pesticide workers usually dissolve methoxychlor in a petroleum-based liquid and apply it as a spray, or they mix it with other chemicals and apply it as a dust (ATSDR, 2002). Methoxychlor has been formulated as wettable powders, dusts, emulsifiable concentrates, ready-to-use products (liquids), and pressurised liquids (US EPA, 2004).

20. According to Götz *et al.* (2008), methoxychlor was used extensively from 1974–1985 as a replacement product for DDT. Between 1986 and 1992, the usage of methoxychlor in the U.S. continuously decreased. After 1992, the use of methoxychlor was heavily reduced. For modelling purposes, Götz *et al.* (2008) estimated the worldwide use of methoxychlor to be three times higher than the use in the U.S. (i.e. worldwide maximum of 8,000 tonnes/year), based on extrapolation factors used for other pesticides, such as trifluralin or DDT. Information on the use of methoxychlor in the U.S. from early 1970s to 1995 is reported in Figure 1 a) and b) (see Appendix). Pesticide uses of methoxychlor in the U.S. were suspended in 2000, and all products were expected to have been voluntarily discontinued by 2004 (US EPA, 2004).

21. It is reported that the use of methoxychlor as a pesticide ceased in most EU countries between the 1970s and 2000 (OSPAR, 2004). In Austria, the volume of methoxychlor used in agriculture was 1 kg/year in 1991–1992 with no use since 1993 (Annex E, 2019). Methoxychlor has been phased out in the European Union since 25 July 2003, with some Member States having put bans in place prior to this (e.g. the substance was not used in Hungary since 1972, in the Netherlands since 1990 and in Austria since 1993 (Annex E, 2019)). The European Agency for the Evaluation of Medicinal Products (EMEA) reported that methoxychlor was not used in veterinary medicines in EU Member States (OSPAR, 2004). According to Langford *et al.* (2012), methoxychlor is no longer registered for use in Norway. The registration of methoxychlor in Canada was withdrawn in 2002, with a phase out period of three years (Annex E, 2019). In Australia, the only methoxychlor product registration was discontinued in mid-1987 (information available from APVMA).[5] Annex E (2019) information from New Zealand indicated that methoxychlor is not approved in New Zealand under the Hazardous Substances and New Organisms Act 1996 (HSNO Act). In Belarus, the use of methoxychlor as an insecticide was banned in 1999 (Annex E, 2019). Egypt indicated that the use of methoxychlor as an insecticide has been banned in Egypt since 1996 by ministerial decree no. 55/1996 (Annex E, 2019). Monaco, Qatar, Republic of Korea, State of Palestine and Thailand do not currently use methoxychlor (Annex E, 2019). Methoxychlor is no longer used in Costa Rica since the permits for the use of this substance were cancelled in 2013. According to Mexico, since August 1991, methoxychlor is a restricted pesticide than can only be used under the supervision of trained and authorized personnel. Furthermore, since the implementation of the "Regulation on the registrations, import and export authorizations, and export certificates for pesticides, fertilizers and toxic or dangerous substances and materials" on 28 March 2005, Mexico does not have data about any application for a registration of pesticides for their environmental evaluation, related to active ingredient methoxychlor. According to

[3] A medicine used to kill parasites that live on the exterior of their host.

[4] EPA in this document refers to US EPA in ATSDR (2002).

[5] This information is taken from the Australian National Environment Protection (Assessment of Site Contamination) Measure 1999, available at the following link.

the PAN International Consolidated list of Banned Pesticides,[6] methoxychlor is banned in the following countries: Guinea, Indonesia, Mauritania, Oman and Saudi Arabia (Annex E, 2019). According to Cindoruk *et al.* (2020), methoxychlor has been banned in Turkey since 2012. According to Kao *et al.* (2019), OCPs have been banned in Taiwan Province of China, since 1975. China indicated that they have stopped the registration of methoxychlor as a pesticide since the 1990s. Currently there is no evidence of use either legally or illegally. Ghana indicated that the use of OCPs such as methoxychlor has been discontinued for several years in Ghana. There are no official data of use of methoxychlor in Ghana.

22. Based on a literature search made for the purpose of drafting this risk profile, recent use of methoxychlor may have occurred in the following countries (non-exhaustive list): Egypt and Turkey.

2.1.3 Releases to the environment

23. Methoxychlor does not occur naturally in the environment (ATSDR, 2002). It is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Since the use of methoxychlor is highly seasonal, the amount that is released to the environment can be expected to be greater during periods of insect control (spring and summer). Smaller amounts of methoxychlor may be released to the environment during its production, formulation, storage, shipment and disposal (ATSDR, 2002). According to the Toxics Release Inventory (TRI), methoxychlor processing facilities listed for 1998 (TRI99 2001) report that the major portion of methoxychlor released to the environment is released to the land (ATSDR, 2002).

24. Methoxychlor is released to soils primarily through its use as an insecticide for agricultural crops, home orchards, and ornamentals. Some methoxychlor may be released to soils through leaks at storage and waste sites. According to TRI (TRI99 2001), three processing facilities in the U.S. reported that no methoxychlor was released to soil in 1999 (ATSDR, 2002). The TRI data should be used with caution since only certain types of facilities are required to report; therefore, the information may not be exhaustive. Methoxychlor has been identified in 46 soil and 11 sediment samples collected from 58 of 1613 National Priorities List (NPL) hazardous waste sites (HazDat, 2002 as cited in ATSDR, 2002). According to TRI data for the year 2018 (US EPA, 2020a), 1.02 tonnes of methoxychlor was released from on-site disposal (or other releases) and 0.02 tonnes of methoxychlor were released from off-site disposal (or other releases). In the U.S., a total of 1.04 tonnes was released in 2018.

25. Release of methoxychlor to the atmosphere occurs mainly as a result of its use as a pesticide. Releases to the atmosphere during production, formulation, and disposal of methoxychlor have been estimated to be 0.5 kg/metric ton produced (Archer *et al.*, 1978). Based on the 193 tonnes of methoxychlor produced in the U.S. in 1991 (Kincaid Enterprises, 1992 as cited in ATSDR, 2002), atmospheric release during production may be estimated to be 96.5 kg/year. According to TRI (TRI99 2001), three processing facilities in the U.S. reported the release of 13 kg of methoxychlor to the air in 1999 (ATSDR, 2002). Götz *et al.* (2008) estimated the worldwide use of methoxychlor to be three times higher than the use in the U.S. (i.e. worldwide maximum of 8,000 tonnes/year). The maximum production of 8,000 tonnes/year is extrapolated from information on production in the U.S. during the late 1970s-early 1980s and since the recent global production figures are not available. Based on a maximum historical estimate of 8,000 tonnes/year of methoxychlor produced worldwide (circa 1975), peak historical atmospheric releases during production were estimated to be up to 4 tonnes/year.

26. Methoxychlor can be released directly to surface water on farms when used to control larvae of insects (Stoltz and Pollock, 1982 as cited in ATSDR, 2002). Methoxychlor was approved for use on cranberries (EPA, 1988b as cited in ATSDR, 2002), which are grown in bogs, and therefore methoxychlor could have been released directly to surface water where cranberries are grown. Methoxychlor may be released to water from runoff from soil, industrial effluents or from leaks at storage and waste sites (ATSDR, 2002). According to Howard (1991), field studies have shown that methoxychlor does not leach significantly in soil. However, groundwater monitoring data suggest that some leaching of methoxychlor may take place (see section 2.3.1 environmental monitoring data). According to TRI (TRI99 2001), three processing facilities in the U.S. reported that no methoxychlor was released to water in 1999 (ATSDR, 2002). Methoxychlor has been detected at 58 of 1613 National Priorities List (NPL) hazardous waste sites. At those 58 sites methoxychlor was identified in 19 groundwater and seven surface water samples (HazDat, 2002 as cited in ATSDR, 2002). Methoxychlor loading into Lake Ontario from the Niagara River was estimated to be 3 kg/year on suspended solids and < 20 kg/year in raw water. Furthermore, loading to Lake Superior from precipitation was estimated to be 120 kg/year (Howard, 1991).

27. Annex E (2019) information from Canada indicated that there are currently no releases of methoxychlor in Canada and information on historical releases is not available. No other information was provided by other Parties on the releases of methoxychlor to the environment while there is some evidence from the literature that the substance may be released to the environment in certain countries (see section “2.1.2 Uses” for further details).

[6] http://pan-international.org/pan-international-consolidated-list-of-banned-pesticides/.

2.2 Environmental fate

2.2.1 Environmental distribution

28. Information on the substance adsorption/desorption, volatilisation and distribution modelling can be found in the Appendix.

2.2.2 Persistence

2.2.2.1 Screening information based on modelling data

29. The aerobic biodegradation potential of the substance was assessed by using BIOWIN v4.10 (U.S. EPA, 2012). The predictions for the structure of methoxychlor are BIOWIN 2: 0.0162, BIOWIN 3: 1.5126 and BIOWIN 6: 0.0063. These values are all well below the screening values, indicating that methoxychlor is not expected to biodegrade rapidly and hence is potentially 'persistent or very persistent' in accordance with the REACH Guidance Chapter R.11 (ECHA, 2017; see further details in the Appendix**)**.

2.2.2.2 Abiotic degradation

Hydrolysis

30. The effect of hydrolysis on the persistence of methoxychlor under environmentally relevant conditions is expected to be negligible. Wolfe *et al.* (1977) investigated the hydrolytic degradation pathways in water at environmentally relevant pH values (3–9), using a standard solution of 1.0×10^{-8} M methoxychlor in distilled water, titrated to pH 5.0 with hydrochloric acid. Five mL aliquots were placed in ampules, sealed and allowed to equilibrate at the target temperature. Rate constants at four temperatures (45, 65, 75, and 85°C) were obtained employing the integrated first-order rate expression using a least-squares analysis of the data. The measured rate constant at 45°C and pH 7 was $3.2 \pm 0.4 \times 10^{-7}$ s^{-1}. Extrapolation of the methoxychlor data obtained at elevated temperatures to 27°C at pH 3–7 gives a first order rate constant of 2.2×10^{-8} s^{-1}, which corresponds to a half-life of 367 days. The calculated half-life of methoxychlor at higher pH (pH 9) was much longer (2100 days at 27°C; calculated from first-order rate constants assuming buffered conditions). Studies with natural waters were carried out with methoxychlor. At 85°C the reaction obeyed first-order kinetics, and there was no detectable change in the rate of hydrolysis in the Oconee River (Athens, GA, USA) water (pH 6.6) or Alabama River (Birmingham, AL, USA) water (pH 7.2) (Wolfe *et al.,*1977).

31. According to Wolfe *et al.* (1977), the major products of methoxychlor hydrolysis at pH 7 are anisoin, anisil and DMDE [2,2-bis(*p*-methoxyphenyl)-1,1-dichloroethylene]. However, the quantification of these hydrolysis products is not reported. Based on a separate experiment (details not reported) and based on structure reactivity relationships and reported rate constants of other compounds, the authors anticipate that anisoin and anisil undergo degradation much faster than methoxychlor. The minor product, DMDE, is more stable to hydrolysis than methoxychlor and might be anticipated to build up to a small extent. Not all experimental details are reported and there are some variations from the current OECD TG 111. Sensitivity of the analytical method and the analytical recoveries are not reported. It is unclear if the test substance was incubated under dark conditions and which method was used for extrapolation to 27°C. The study is therefore reliable with restrictions. According to Katagi (2002) as cited in HSDB (2009), the hydrolysis half-lives of methoxychlor in distilled water varies considerably with pH, with half-life values at 27°C of 1 year (pH 7) and 5.5 years (pH 9). The half-lives from the Katagi (2002) study (as cited in HSDB (2009)) are in agreement with the Wolfe *et al.* (1977) study.

Photolysis

32. Remucal (2014) suggested that methoxychlor undergoes negligible direct photolysis because it does not absorb light in the solar spectrum. Experimental evidence suggests that methoxychlor is susceptible to dissolved organic matter-sensitised indirect photolysis. The addition of dissolved organic matter from natural waters has been reported to accelerate the photodegradation of methoxychlor in water when irradiated at wavelengths >290 nm (Remucal, 2014).

33. Zepp *et al.* (1976) developed a laboratory scale study to assess the direct photolysis of pesticides in the aquatic environment. The investigations included light-induced degradation of methoxychlor in distilled and natural water samples. Assuming first-order kinetics, the direct photolysis half-life of methoxychlor in natural waters was much more rapid (2 to 5 hours; solution in sealed quartz cells exposed to midday May sunlight, latitude 34°N) than that in distilled water (4.5 months; assuming 12-h days exposure and latitude 40°N), suggesting that photochemical processes other than direct photolysis can cause more rapid degradation of methoxychlor in some natural aquatic environments. The major product of methoxychlor photolysis in distilled water was DMDE (Zepp *et al.*, 1976). This study indicates degradation in specific conditions under influence of sunlight. Although photolysis rates of methoxychlor seem moderate, photolysis is not expected to contribute to the degradation significantly as photolysis only takes place in the top layer of the water column.

34. Dry, thin films of methoxychlor exposed to direct sunlight readily decomposed. In 6 days, 6.6% of a 10 mg sample of methoxychlor disappeared. After 12 days, 91.4% of a 5 mg sample of methoxychlor disappeared, but the total amount lost was approximately the same over the 12-day exposure period (NRCC, 1975). Films of pure compound on solid surfaces absorb sunlight more rapidly than dilute solutions (Zepp *et al*., 1976). ASTDR (2002) reported that based on photodegradability of methoxychlor in water and the photodegradation of a structural analogue (ethoxychlor) in soil (Coats *et al.*, 1979 as cited in ATSDR, 2002), photodegradation is likely to occur in soil, but only at the very surface. Because methoxychlor is mostly found in the upper layer of soil (top 5 cm of soil on which it was applied (Golovleva *et al.*, 1984 as cited in ATSDR, 2002), photochemical degradative processes would probably be more important for methoxychlor applied to crops. However, soil monitoring data (Abong'o *et al.*, 2015; Bolor *et al.*, 2018; Thiombane *et al*., 2018) indicated that methoxychlor was found in the plough layer (0-30 cm) and groundwater monitoring data (US EPA, 1987; Plumb, 1991; Helou *et al.*, 2019; Affum *et al.*, 2018) suggest that some leaching in soil may take place. According to Katagi (2004), the depth of light penetration (photic depth) in soil is not larger than few millimetres. This is confirmed by the draft OECD test guideline on phototransformation of chemicals on soil surfaces (2002), where the test protocol refers to the preparation of soil thin-layers of about 2 mm thickness in order to measure phototransformation on irradiated dry soil surfaces. As a consequence, methoxychlor photodegradation in soil at depths below a few millimetres is not expected to happen.

35. Methoxychlor may undergo photolysis on soil surfaces, which is based on studies reporting the photolysis of dry methoxychlor films exposed to sunlight and on the photodegradation of a structural analogue (ethoxychlor) in soil. The effect of hydrolysis and photolysis in water and soil on the persistence of methoxychlor in the environment is expected to be negligible.

2.2.2.3 Biotic degradation

36. Degradation rates of methoxychlor in water, sediment and soil are shown to be impacted by the relative presence or absence of oxygen. The aerobic degradation rate is slow, possibly negligible, but the anaerobic degradation rate is faster (OSPAR, 2004). As is discussed in the following paragraphs, this is likely to be explained by the biotic and abiotic processes, and microbial species prevailing under anaerobic conditions.

37. Muir and Yarechewski (1984) studied ^{14}C-methoxychlor (^{14}C-ring-labelled) degradation in water-sediment systems (water-sediment ratio: 20:1; weight of pond or lake sediments: 10 g dw) under aerobic and anaerobic laboratory conditions using respirometer flasks. Lake (79% clay, 21% silt and 6.0% organic matter) and pond (75% clay, 24% silt, 6.3% organic matter; pH=7.6) sediments were incubated in a controlled environment (22.5°C) using a photoperiod of 16-hour light and 8-hour darkness. Nitrogen aerated flasks were darkened by covering them with aluminium foil. Respirometer flasks were connected to a manifold which delivered air (CO_2 free grade) or nitrogen presaturated with water and included traps for collecting volatiles that were routinely changed up until and including day 224. Flasks were removed for analysis at intervals over a 448-day period. It was observed that methoxychlor added to sediment-water mixtures (methoxychlor concentration of 0.1 µg/mL (or 100 µg/L) in each flask, from diluting 0.1 mL prepared in acetone) was slowly biodegraded under aerobic conditions (half-lives (assuming first-order kinetics) were 115.9±74.1 days and 206.3±186.8 days for pond and lake sediment, respectively; Eh 220 to 464 mv) and degraded more rapidly anaerobically with half-lives of <28 days for both lake and pond sediment (Eh less than -50 mv). Large confidence limits on the half-lives likely reflect the small number of data points (generally 6 points). These results suggest that the degradation of methoxychlor under aerobic conditions, e.g. on suspended sediment or at the sediment-water interface, is relatively slow. Under anaerobic conditions, rapid breakdown of methoxychlor can be expected with dechlorinated methoxychlor (DMDD) and mono- and di-hydroxy degradation products being the major residues present in sediments. However, several deviations from the OECD TG 308 have been noted.

38. The main deviations from the OECD TG 308 are as follows: the water-sediment ratio exceeded the recommended range of between 3:1 and 4:1, the water was not collected from the same site as the sediment (dechlorinated water was used), the test duration exceeded 100 days and it is unclear if microbial activity was in decline, the aerobic test was not performed under totally dark conditions and it is unclear if a solvent control was included in the test. It is also unclear if the pH of the water increased due to delivery of CO_2 free air to the respirometer flasks. Due to these deviations from the test guideline OECD TG 308 (which was unavailable at the time of the test), the study is considered reliable with restrictions. The presence of non-extracted methoxychlor from sediment was not taken into account in the calculation of the half-lives and the calculated half-lives may not therefore represent the most conservative case for methoxychlor.

39. According to WHO (2004), methoxychlor residues may persist in top soil for up to 14 months. A half-life in soil of 120 days was reported by Chen (2014) and Wauchope *et al.* (1992). However, the temperature is unknown and further detailed information was not available in both citations. It seems that the half-life of 120 days was derived from three other studies (Wauchope *et al.*, 1992) having the following half-lives for methoxychlor: 7–60 days, 170 days and 151–210 days. However, it was only possible to review the original reference for the result of 151–210 days by Guth *et al.* (1976). Guth *et al.* (1976) reported half-lives in soil of 151 (soil 1) and 210 days (soil 2) for methoxychlor (calculated by the first-order rate law and obtained in the laboratory at 22±2°C; at pH 4.8 (soil 1)

and pH 6.5 (soil 2); soil type: sandy loam with 1% OC (soil 1) and 2.2% OC (soil 2)). German standard soils were incubated with the pesticides in Erlenmeyer flasks closed with cotton wool plugs to allow air exchange. As details of the study method are missing in Guth *et al.* (1976), the results of this study should be considered with caution. Based on the limited information available for half-lives in soil, cited above, it is not possible to draw a definitive conclusion as to whether methoxychlor is persistent in soil. The half-life in soil of 210 days has been used as input data for the distribution modelling (Mackay Level III Fugacity Model) and for the OECD *P*ov and LRTP Screening Tool.

2.2.2.4 Monitoring data

40. After its ban in September 2006 in the EU and based on the Water Framework Directive (WFD) factsheet,[7] methoxychlor was quantified in 1.26 % of the 4201 inland surface water samples between 2006 and 2014 representing four countries (Lettieri and Marinov personal communication, May 2020). Fifty-three of the 4201 samples showed quantified concentrations of methoxychlor above the Predicted No Effect Concentration (PNEC) value of 0.0005 µg/L (or 0.5 ng/L; derived by INERIS) (limit of detection (LOD) of 0.007 µg/L (or 7 ng/L) and limit of quantification (LOQ) of 0.02 µg/L (or 20 ng/L)). Regarding the monitored fractions in these quantified samples, only two samples are indicated as 'dissolved fraction' while all other quantified samples are 'whole water'. Based on the available information, no use that is exempted from the general ban had been authorised in the respective EU Member States after September 2006 and may therefore be linked with these findings. The European Working Group on Standards (WG-S) concluded that data quality of these monitoring data for methoxychlor was not sufficient to propose it as a candidate for quality standard derivation (due to the large number of unquantified results and limited number of Member States for which data was available). In Slovakia, chlorinated pesticides (such as methoxychlor) have been detected in the drinking water at the level of 10 µg/L (or 10,000 ng/L) which is higher than the EU general limit in drinking water for individual pesticides of 0.1 µg/L or 100 ng/L (EC, 1998). This level reflects a past contamination to these pesticides which were used in Slovakia for agricultural purpose in the 80–90s (Slovak Republic, 2016).

41. Monitoring data[8] from France indicated that methoxychlor was detected in 73 of the 202,923 samples taken from surface waterbodies between 2000 and 2018 at concentrations above the method detection limits. Methoxychlor was measured in one or more surface water samples from France in 2005, 2007, 2008, 2009, 2010, 2011, 2013, 2014, 2015, 2016, 2017 and 2018. The measured concentrations were in the range of 0.005–0.269 µg/L (or 5–269 ng/L), and the corresponding method detection limits ranged from 0.001–0.3 µg/L (or 1–300 ng/L). The highest measured concentration following phase out of methoxychlor in France (in 2006) was 0.269 µg/L (or 269 ng/L) (in 2013). In French groundwaters, methoxychlor was detected in 30 of the 118,563 samples taken from 19,428 monitoring sites[9] between 1990 – 2018. The majority of these sites are from tap water survey network. Methoxychor was quantified from 1998 to 2018 at least in one site every year except in 2000, 2002, 2003, 2006-2009, 2015. The measured concentrations were in the range of 0.001– 0.089 µg/L (or 1– 89 ng/L), and corresponding method detection limits ranged from 0,00001–100 µg/L (or 0.01–100,000 ng/L). The highest concentration (0.089 µg/L (or 89 ng/L)) was measured after phasing out of methoxychlor, in 2010.

42. In addition, Annex E (2019) information from Canada indicated that methoxychlor was detected in 16 of the 1582 samples taken from surface waterbodies between 2000 and 2015 at concentrations above the method detection limits. Methoxychlor was measured in one or more surface water samples from Canada in 2001, 2002, 2007, 2008, 2009, and 2011. The measured concentrations were in the range of 0.027–7.9 ng/L, and the corresponding method detection limits ranged from 0.0916-7.9 ng/L. The highest measured concentration following phase out of methoxychlor in Canada (in 2006) was 5.25 ng/L (in 2009). These data indicate that methoxychlor continued to be detected in European and Canadian surface waterbodies (and in French groundwaters) for years after it was phased out (September 2006 in the EU and January 2006 in Canada), thus providing some evidence that the substance is persistent in the water compartment. However, presence of methoxychlor in European and Canadian surface waterbodies can also result from its long-range transport.

43. Three chemicals, methoxychlor, endosulfan and pentachloroanisole were found in an Arctic lake at concentrations in the range of 0.017–0.023 ng/L (Muir *et al.*, 1995b as cited in AMAP, 1998). Gao *et al.* (2019) monitored methoxychlor in surface seawater (from 33°N to 83°N) in a region covering the North Pacific to the Arctic Ocean. Samples were collected from 12 July to 23 September 2016 and from 27 July to 7 October 2017. Methoxychlor concentrations in the North Pacific Ocean were <MDL[10]–0.54 ng/L (mean 0.13±0.13 ng/L; MDL of 0.01 ng/L) and in the Chukchi Sea <MDL–0.38 ng/L (mean 0.15±0.11 ng/L). The presence of methoxychlor in an Arctic lake water and in the Arctic Ocean provides some evidence that methoxychlor is persistent in marine and freshwater compartments. This conclusion is supported by findings in European and Canadian waterbodies years after

[7] The WFD factsheet for methoxychlor is available at the following link: https://circabc.europa.eu/webdav/CircaBC/env/wfd/Library/working_groups/priority_substances/2a%20-%20Sub-Group%20on%20Review%20of%20Priority%20Substances%202014%20start/Monitoring%20based%20exercise/Factsheets/Methoxychlor_draft%20Factsheet_annex%20monitoring%20report.pdf.

[8] Link to database : http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie.

[9] Link to database: https://ades.eaufrance.fr/Recherche/Index/QualitometreAvance?g=933c15.

[10] Method Detection Limit (MDL).

it was phased out. However, the presence of methoxychlor in the Arctic lake and seawater can also result from its long-range transport.

44. Pinto *et al.* (2016) found methoxychlor in sediment core from the upper part of Óbidos lagoon (Portugal). Sediment samples were collected in November 2013. Methoxychlor was found at concentrations ranging from 21.8 to 89 ng/g dw in the core sediment between 16 and 40 cm depth (Pinto personal communication, September 2019). As the sedimentation rate is not available, it was not possible to date the concentrations found below 14 cm depth. However, the presence of methoxychlor in sediment core below 14 cm provides evidence of high inputs in the past (before its phase out in the EU in 2006) and that the substance may be persistent in some anaerobic sediments. Sediment is expected to be anaerobic at this depth (16–40 cm). The fast biodegradation under anaerobic conditions reported in the laboratory study of Muir and Yarechewski (1984) (see section "Biotic degradation") is not supported by the field study of Pinto *et al.* (2016). Considering the results of Pinto *et al.* (2016), the substance may be persistent in some anaerobic sediments.

45. Duodu *et al.* (2017) found methoxychlor in sediment samples collected between 2014 and 2015 from the Brisbane River estuary of Southeast Queensland in Australia. The sediment samples were collected in the months of June (winter), September (spring), December (summer) 2014, and May (autumn) 2015; thus, spanning both dry and wet seasons. A total of 22 sampling points were selected along a 75 km stretch of the river from the mouth. Grab sediment (0–3 cm depth) samples were collected with laboratory recoveries > 85% for methoxychlor. The average concentrations of methoxychlor were consistent among the four physio-geographical areas (namely rural, residential, commercial and industrial; average: 4.3±0.2 ng/g; range: 4.1–4.8 ng/g; detection frequency: 100%; the MDL ranged between 0.06 and 2.3 ng/g with relative standard deviation (RSD) < 10%). It is likely that the measured concentrations underestimate the real concentrations of methoxychlor because aged sediments will sorb the substance more strongly than a spiked sample generated for recovery purposes in the laboratory. These data suggest that the input of methoxychlor was historical in nature. The Brisbane River estuary is micro-tidal, with limited inflow of freshwater. Due to the relatively low freshwater inflow, strong tidal mixing acts as a dominant mixing mechanism in the estuary, thereby causing resuspension of fine sediment. This study provides evidence that methoxychlor is persistent in sediment, which in this study is expected to have been aerobic (0–3 cm depth from a river). In Australia, the only methoxychlor product registration was discontinued in mid-1987 (information available from APVMA).[11]

46. Annex E (2019) information from Canada indicated that methoxychlor was detected in 48 of the 301 samples of Great Lakes sediments (0–15 cm depth but most of the samples were taken from surface sediments) between May 2013 and September 2017. The mean concentration of these 48 detections was 3.7 ng/g (with concentrations ranging from 0.075 ng/g to 36 ng/g). These data indicate that methoxychlor continued to be detected in Canadian sediments years after its phase out (January 2006), thus supporting evidence of its persistence in the sediment compartment.

47. Thiombane *et al.* (2018) studied contamination levels of OCPs in urban and rural soils from central and southern Italy. Topsoil samples (n= 148; 0–20 cm top layer) were collected from early April to the end of September 2016 in urban and rural areas throughout 11 regions from the centre to southern Italy. In each region, the main urban areas and the nearest rural areas (where most of the land is devoted to agricultural activities) were selected. Methoxychlor was found in the range of n.d.–53.23 ng/g (mean value: 3.64 ng/g) in urban areas and n.d.–521.79 ng/g (mean value: 10.96 ng/g) in rural areas (LOD of 0.025 ng/g). According to Thiombane *et al.* (2018), methoxychlor was found to represent 12.17% of the total OCPs detected in soils from urban areas, which the authors state was likely to be related to recent applications (particularly in Apulia), while the use of the substance has been banned in plant protection products since 2003 and in biocidal products since 2006 in the European Union. However, based on the available information, no use that is exempted from the general ban had been authorised in Italy after September 2006, i.e. the use of methoxychlor (as plant protection product or biocide) due to special circumstances of a danger that cannot be contained by other means and provided that the authorisation is notified to the European Commission and the other Member States. These data indicate that methoxychlor continued to be detected in European soils years after its phase out, thus providing some evidence that the substance may be persistent in some aerobic soils. However, presence of methoxychlor in European soils can also result from its long-range transport.

2.2.2.5 Summary on persistence

48. Modelling data (BIOWIN 2, 3 and 6) indicate that methoxychlor is not expected to biodegrade fast, and hence is potentially persistent. It is noted that the available hydrolysis studies indicate that hydrolysis is expected to be negligible in the environment. Although photolysis rates of methoxychlor seem moderate, photolysis in water is not expected to contribute to the degradation significantly as photolysis only takes place in the top layer of the water column. In addition, methoxychlor may undergo photolysis on soil surfaces, which is based on studies reporting the photolysis of dry methoxychlor films exposed to sunlight and on the photodegradation of a structural analogue (ethoxychlor) in soil.

[11] This information is taken from the Australian National Environment Protection (Assessment of Site Contamination) Measure 1999, available at the following link.

49. The degradation half-life of methoxychlor in one of two aerobic sediments exceeds the persistence criterion (115.9±74.1 days for pond and 206±186.8 days for lake (half-life > 6 months; Muir and Yarechewski (1984)). In the same study, methoxychlor degraded more rapidly anaerobically with half-lives of < 28 days for both lake and pond sediment. However, these calculated half-lives may not represent the most conservative case for methoxychlor since non-extracted methoxychlor from sediment was not taken into account. Persistence of methoxychlor in aerobic and anaerobic sediments is supported by monitoring data. Methoxychlor was found in sediment samples from Portugal, Australia and Canada several years after methoxychlor was banned in these countries (Pinto *et al.*, 2016; Duodu *et al.*, 2017 and Annex E, 2019 information from Canada). Based on a weight-of-evidence approach, the results from the laboratory study and monitoring data indicate that methoxychlor is persistent in aerobic sediments and may be persistent in some anaerobic sediments. Monitoring data have indicated that methoxychlor continued to be detected in surface waterbodies in Europe and Canada, and in French groundwaters, years after it was phased out, thus providing some evidence that the substance is persistent in water. Monitoring data from an Arctic lake and surface seawater in a region covering the North Pacific to the Arctic Ocean, further indicate that the substance may be persistent in the surface water and marine water compartments. Soil laboratory studies reported degradation half-lives in the range of 7-210 days (Chen, 2014; Wauchope *et al.*, 1992 and Guth *et al.*, 1976). However, due to study limitations it was not possible to draw a definitive conclusion as to whether methoxychlor is persistent in soil. Methoxychlor was found in soil samples from Italy several years after methoxychlor was banned in the EU (Thiombane *et al.*, 2018). Based on a weight-of-evidence approach, the results from the laboratory studies and monitoring data indicate that methoxychlor may be persistent in some aerobic soils. However, the presence of methoxychlor in the surface water, seawater and soil mentioned above can also be a result of long-range transport.

2.2.3 Bioaccumulation

2.2.3.1 Screening information based on modelling data

50. Methoxychlor is a strongly hydrophobic substance with an experimental log K_{ow} value of 5.08 (Karickhoff *et al.*, 1979). The substance has an estimated log K_{ow} value of 5.67 (KOWWIN v1.68 (US EPA, 2012) which is consistent with the experimental value. Both experimental and estimated log K_{ow} values indicate a bioconcentration potential of methoxychlor in aquatic organisms (log K_{ow} >5).

51. The predicted BCF and BAF values are reported in the Appendix. The BAF value (9001 L/kg) predicted by the Arnot-Gobas method (upper trophic) suggest bioaccumulation potential of methoxychlor in aquatic organisms (BAF > 5,000).

2.2.3.2 Bioconcentration and bioaccumulation studies in aquatic organisms

52. The BCF values for methoxychlor vary largely between different aquatic species as a result of species differences in the capacity to metabolise and excrete methoxychlor (BCFs are in the range of 667–8,300). The maximum laboratory derived BCF value for methoxychlor is 8,300 in fathead minnow, as presented in Veith *et al.*, 1979.

53. Veith *et al.* (1979) developed a method for estimating BCFs using a laboratory experiment exposing adult fathead minnows (*Pimephales promelas*) (~6 months old) to a non-lethal dose of methoxychlor (included in an acetone solution) in a continuous-flow system at 25±0.5°C. The test water came from Lake Superior. The arithmetic mean of the pH was 7.49±0.15. Dissolved oxygen varied with the number of fish in the tanks and was maintained > 5 mg/L. The fish were fed daily with frozen brine shrimp. The concentration of the substance in the water was measured daily (methoxychlor mean exposure/concentration in water: 3.5 µg/L). Thirty minnows were placed in the tank with the test substance (five fish in the control tank). Five fish were analysed after 2, 4, 8, 16, 24 and 32 days of exposure to methoxychlor. The accuracy of the analytical methods was examined by determining the recovery of known amount of the chemical in water or tissue. The experimental procedures were adjusted so that at least 90% of the added substance was recovered. The water and tissue concentrations were not corrected for recovery in the calculation of bioconcentration factors. The steady-state bioconcentration factor was calculated from the concentrations in fish and water at day 32 of exposure. A BCF value for methoxychlor of 8,300 was reported. The study authors do not confirm whether steady-state was reached at day 32. The BCF may have been higher than 8,300.

54. It was noted by the authors that at early stages of this study, occasional spawning was observed, and spawning tiles were used in the exposures to reduce the excitability of the fish. Some experimental details are missing so it is difficult to fully assess the validity of this study. It is unclear if the following validity criteria of the OECD TG 305 have been met: the concentration of the test substance was maintained within ± 20% of the mean of the measured values during the uptake phase and the mortality of fish in both control and treated fish is less than 10% at the end of the test. In addition, no depuration phase was performed in the test and it is most likely that the BCF for methoxychlor was not lipid and growth corrected. However, the BCF result is considered to be reliable with restrictions since the experiment follows the key principles of the OECD TG 305 and methoxychlor was tested in a flow-through system at a concentration below its water solubility.

55. The OECD Guidelines for Testing of Chemicals (TG 305) outlines a method for determining a chemical's bioaccumulation in fish by exposing test fish to either food or water spiked with the test substance. In a validation study of a ring test for the OECD TG 305 dietary exposure test, the results from eight different laboratories were assessed (OECD, 2012a and OECD, 2013).[12] The studies were conducted using rainbow trout (*Oncorhynchus mykiss*) with one laboratory conducting a further study using carp (*Cyprinus carpio*) (OECD, 2012a). BCF values for methoxychlor were derived from the dietary test data as follows: the equivalent uptake rate constant of methoxychlor from water (k_1) was estimated using the "best" methods identified from a review of 13 different existing methods from the literature. Inter-laboratory kinetic BCF values were then estimated as the ratio of the estimated k_1 value from seven different methods of calculation to the overall mean measured depuration rate constant (k_2), or to the mean measured growth corrected depuration rate constant (k_{2g}). Using the k_{2g} value, estimated BCF values for methoxychlor from six laboratories ranged between 3,335 L/kg and 5,926 L/kg for rainbow trout (mean = 4,421 L/kg), and 1,006 L/kg to 2,015 L/kg for carp (mean = 1,537 L/kg; one lab), using a 3% feeding rate of wet body weight per day (OECD, 2012a). The mean estimated lipid-normalised (5% lipid) and growth-corrected BCF for rainbow trout is 2,941-6,991 (excluding the data from laboratory 5 which had some uncertainties) and for carp is 667-1,867 (Environment Agency, 2014). Estimated BCF values using a 1.5% feeding rate were lower compared with values estimated using the 3% feeding rate (not growth corrected; OECD, 2013). The mean growth-corrected, lipid-normalised biomagnification factor (BMF_{gL}) for methoxychlor in rainbow trout estimated from the ring tests is 0.14 with a relative standard deviation of 71 % for a feeding rate of 3% and 0.022 from a single test using a feeding rate of 1.5% (OECD, 2017). It is noted that several highly bioaccumulative substances exhibit in fish dietary tests BMFs $<<$ 1 and such results are not directly indicative of lack of field biomagnification potential.[13]

56. Inoue *et al*, 2012 performed dietary exposure tests according to OECD TG 305. Common carp were exposed via diet to a mixture of methoxychlor, musk-xylene and o-terphenyl and the reference substance hexachlorobenzene. The lipid- and growth-corrected BMF for methoxychlor was 0.034 ± 0.001. The paper also cites a steady-state, lipid normalised BCF of 810 for methoxychlor, obtained from Japanese Chemical Substances Control Law test reports.

57. Renberg *et al*. (1985) studied the bioconcentration potential of methoxychlor in a bivalve *Mytilus edulis*. A continuous-flow system was used for the duration of the 21-day study. Bivalves are known to close their valves under unfavourable conditions, which occasionally may bias the results. In order to overcome this disadvantage, an internal standard (pentachlorobenzene) was added to the water simultaneously with methoxychlor. Although there is a risk for interactive effects, the authors stated that unexpected variations in the uptake can be compensated by relating the concentration of the test substance to the concentration of the internal standard in the organisms (benchmark approach). The bivalves were collected at a depth of 1–3 m in the Baltic bay Tvären and were kept up to 8 months at 10°C. Mussels with an approximate size of 3.5 cm were selected and 20 individuals were spread over each of the two glass plates which were transferred to the aquarium used for the experiment. The stock solution consisting of an acetone solution of the test substance (level of purity: 100%) was mixed with brackish water using a magnetic stirrer. Recovery experiments for both water and organisms showed recoveries over 85% for the test substances. Mussels were fed with unicellular green algae. A BCF value of 12,000 was observed for methoxychlor at day 21. A steady-state was not completely reached at the end of the 21 days suggesting that the BCF value could be even higher than 12,000 for *Mytilus edulis*. Additional static tests performed over 8 days gave BCF values of 8,020 and 8,400 but steady-state was not reached over such a short time period. Experimental details are missing (e.g. water quality parameters, temperature) so it is difficult to fully assess the validity of this study. Since a reliable experimental set up was used and methoxychlor was tested in a flow-through system at a concentration below its water solubility, the results can be used to indicate that the BCF for *Mytilus edulis* is > 5,000.

58. Anderson and DeFoe (1980) conducted an experiment exposing stoneflies, caddis-flies, isopods, snails and bullheads to methoxychlor in a flowing-water test system for 28 days and unfiltered lake water was used under realistic natural conditions (temperature, pH, dissolved oxygen etc.). Methoxychlor BCFs were determined in the snails *Physa integra* with an average BCF of 6,945 (range of 5,000 to 8,570 depending on the concentration tested). It is unclear if steady-state was achieved at the end of the experiment (at day 28). The study aimed to determine toxicity of methoxychlor as well as bioaccumulation and although no mortality of *Physa integra* was observed, it is not clear whether there were any sub-lethal effects and thus whether the test concentration was too high. Therefore the BCF of 6,945 is only used as supporting information.

59. The environmental consequences of the combination of the bioaccumulation potential of methoxychlor with a high toxicity (NOAEL as low as 0.6 mg/kg bw/day (Aoyama *et al.*, 2012)) and high ecotoxicity (NOECs[14] below 1.3 µg/L (or 1,300 ng/L) for fish and aquatic invertebrates (Anderson and DeFoe (1980), HSDB (2009) and US EPA (2019)) and HC_5 of 0.37 µg/L or 370 ng/L for freshwater arthropods (Maltby *et al.* (2005))) gives reason for concern.

[12] OECD (2012) considers the results from eight laboratories (including those in UK, Germany, France, USA, Canada, Norway, Switzerland, Japan).

[13] Please, see further discussion on the difficulty to interpret BMF results from a dietary bioaccumulation test in ECHA Guidance (ECHA, 2017), section R.11.4.1.2.3.

[14] No Observed Effect Concentrations (NOEC).

60. Methoxychlor has been reported in the Arctic in biota samples (terrestrial, avian and marine; Vorkamp *et al.*, 2004 and Savinov *et al.*, 2011) and in Antarctica (in marine biota samples and in milk of elephant seals (Filho *et al.*, 2009). In addition, methoxcyhlor has been found in human breast milk (Damgaard *et al.,* 2006).

2.2.3.3 Toxicokinetic and metabolism studies

61. **Absorption:** Studies in mammals (mice, rats and goats) indicate that methoxychlor is well absorbed by the gastrointestinal tract and to a lesser extent by the skin (ATSDR, 2002). However, some of the data from animal studies come from ruminant animals, which may have limited relevance to humans and other non-ruminant species.

62. **Distribution**: Studies in mammals (dogs, rats, sheep and goats) (as reported in ATSDR, 2002) indicate that once in the bloodstream, methoxychlor appears to distribute to most tissues of the body, with highest levels usually found in fat (however, levels of methoxychlor do not remain elevated for very long after exposure).

63. **Metabolism:** Studies in mammals (rats, mice, goats) (as reported in ATSDR, 2002) indicate that methoxychlor is metabolised rapidly by the liver and neither the parent compound nor the metabolites tend to accumulate in fat or other tissue. The metabolism of methoxychlor has been fairly well studied *in vitro* and *in vivo* in animals and with human liver microsomes. Both sets of data indicate that methoxychlor undergoes demethylation to form phenolic derivatives, with dechlorination and dehydrochlorination reactions occurring to a lesser extent.

64. **Elimination:** Studies in mammals (rats, mice, goats) (as reported in ATSDR, 2002) indicate that most of the ingested dose of methoxychlor is eliminated in the feces via biliary excretion of metabolites. Urinary excretion contributes to a lesser extent (approximately 10% of the total administered dose as indicated in mouse studies). Methoxychlor and/or its metabolites have been detected in milk following oral exposure of animals to methoxychlor during lactation. The toxicokinetics of methoxychlor in humans is expected to be similar to the toxicokinetics of methoxychlor observed in animals (ATSDR, 2002).

2.2.3.4 Conclusion on bioaccumulation

65. Available studies suggest that the BCF values of methoxychlor vary largely between different aquatic species (BCFs in the range of 667–8,300). Laboratory studies indicate that methoxychlor has a bioaccumulation potential in some fish species with BCF values > 5,000. Supporting information in a bivalve (BCF of 12,000) and in snails (BCF in the range 5,000 to 8,570) also indicate a bioaccumulation potential in aquatic invertebrates. The potential for bioaccumulation is supported by experimental and estimated log K_{ow} values > 5 that predict that bioaccumulation of methoxychlor in aquatic organisms is likely. Combination of the bioaccumulation potential of methoxychlor with a high toxicity and high ecotoxicity gives reason for concern. Toxicokinetic and metabolism studies would suggest that methoxychlor does not accumulate in mammals.

2.2.4 Potential for long-range environmental transport

2.2.4.1 Screening of physicochemical properties

66. The experimental Henry's Law constant is 2.06 x 10^{-2} Pa.m^3/mol at 25°C (US EPA, 2012) and the estimated vapour pressure for methoxychlor is 5.56 ×10^{-3} Pa at 25 °C (EPI Suite, MPBPVP v1.43; US EPA, 2012), both values suggest a low potential for volatilisation of methoxychlor to the atmosphere. The reliability of the predicted vapour-pressure can be considered good because it is within the parametric domain of the model and there are structurally similar compounds in the training set. However, concentrations of methoxychlor found in remote regions suggest that atmospheric transport has occurred. Vapour-phase methoxychlor will be degraded in the atmosphere primarily by reaction with photochemically-produced hydroxyl radicals. The rate constant for the vapour-phase reaction of methoxychlor with hydroxyl radicals has been estimated as 5.4 ×$10^{-11}$$cm^3$/molecule·s at 25°C (AOPWIN v1.92; US EPA, 2012). The estimated half-life of methoxychlor in air is 2.4 hours in continuous light, or 0.2 day based on a 12-h photoperiod with 1.5 × 10^6 OH/cm^3 (AOPWIN v1.92; US EPA, 2012). There is some uncertainty associated with the reliability of the AOPWIN prediction since although the substance is within the parametric domain of the model, the substances in the training set of the model are structurally different to methoxychlor. Furthermore, it has been shown for the structural analogue DDT that AOPWIN overestimates the reactivity with OH radicals of large molecules. For DDT, AOPWIN v1.92 gives a 2nd-order rate constant of 3.435 × 10^{-12} cm^3/(molecule·s). In contrast, Liu *et al.* (2005) found a measured value of 5 × 10^{-13} cm^3/(molecule·s) for DDT, which is by a factor of 7 lower than the AOPWIN estimate. Because the sorbed fraction is likely to be resistant to atmospheric oxidation, the AOPWIN half-life value based on reaction with hydroxyl radicals is most probably an underestimation of the half-life in air.

67. Modelling estimates predict that a portion of methoxychlor in air may be sorbed to particulates, which may increase its residence time and potential for long-range transport. Model estimations of the fraction of methoxychlor sorbed to atmospheric particles range from 0.5 to 22% (AEROWIN v1.00). The reliability of the modelled value can be considered good because the input values for vapour pressure and log K_{oa} (applied in AEROWIN) are within the applicability domains of the respective models (EPI Suite, MPBPVP v1.43 and KOAWIN v. 1.10). As cited in ATSDR (2002), Kelly *et al.* (2004) describe that methoxychlor is expected to exist in both the vapour and particulate phases (bound to particulate matter) and, to a small degree, in the vapour phase in the atmosphere. The residence time

and dispersion of methoxychlor in air is, therefore, a function of particle size, windspeed, and precipitation (ATSDR, 2002).

68. Based on monitoring data, the majority of methoxychlor in the atmosphere may be removed by wet or dry deposition processes with a residence time of <1 month (Hoff *et al.*, 1992 as cited in ATSDR, 2002). However, evidence of wide dispersion of methoxychlor in the atmosphere by its detection in Canadian Arctic snow suggests that some methoxychlor may remain in air for extended periods of time (Welch *et al.*, 1991). Methoxychlor has been frequently detected in rain (Strachan 1985, 1988 as cited in ATSDR, 2002). In a 6-year study (1986–1991) conducted in the Great Lakes Region, the mean annual concentration of methoxychlor in rain was 2.4 ng/L (Chan *et al.*, 1994 as cited in ATSDR, 2002). These data suggest that wet deposition processes significantly contribute to the removal of methoxychlor from the atmosphere. However, wet deposition of methoxychlor will depend upon the amount of precipitation and will vary from year to year. Dry deposition due to gravity will also act to remove methoxychlor from air (ATSDR, 2002). In the Great Lakes region, dry deposition of chlorinated pesticides was estimated to be 1.5–5.0 times as great as wet deposition (Eisenreich *et al.*, 1981 as cited in ATSDR, 2002). The OCPs, endosulfan, methoxychlor, and pentachloroanisole, were detected in an Arctic lake. Muir *et al.* (1995b; as cited in AMAP, 1998) found all three compounds present at similar concentrations (0.017–0.023 ng/L) in water from Peter Lake, a large oligotrophic lake near Rankin Inlet (Northwest Territories (NWT)). The presence of these compounds is consistent with their presence in Arctic air as reported in 1997 (Barrie *et al.*, 1997 as cited in AMAP, 1998).

69. If released to soil, it is expected that wind erosion of the upper layers of the soil can transport methoxychlor that has not degraded and has been incorporated to soil particles.

2.2.4.2 Long-range transport model predictions

70. Overall persistence (*P*ov) and the potential for long-range transport (LRTP) of methoxychlor was estimated using the OECD *P*ov and LRTP Screening Tool (Wegmann *et al.*, 2009; see further details in the Appendix). The results obtained for methoxychlor suggest that it has a low LRTP: characteristic travel distance (CTD) of 498 km, *P*ov of 303 days and transfer efficiency from air to surface media of 0.02 %. However, the *P*ov of methoxychlor (303 days) is higher than the *P*ov of α-HCH (195 days). The results from this modelling are associated with uncertainty due to the uncertainties of the input parameters. Muir *et al.* (2004) and Hoferkamp *et al.* (2010) suggest that the presence of methoxychlor in the Arctic can be explained by atmospheric transport via the gas phase or on dust during periods without rainout events and lower rates of photodegradation than predicted from standard assumptions, due to transport in periods of lower photolytic activity. Muir *et al.* (2004) note that the OECD *P*ov and LRTP Screening Tool and other similar models assume continuous low rates of precipitation which are not reflective of conditions found in all regions during all seasons.

71. The experimental log K_{ow} (5.08), log K_{oa} (10.48) and log K_{aw} (-5.081) values for methoxychlor suggest high potential to reach the Arctic and to accumulate in the Arctic human food chain according to the criteria cited by Brown and Wania (2008). Indeed, the area of elevated Arctic Contamination and Bioaccumulation Potential (AC-BAP) selected by Brown and Wania (2008) comprises the following criteria: log $K_{ow} \geq 3.5$; log $K_{oa} \geq 6$; $0.5 \geq$ log $K_{aw} \geq -7$; log $K_{aw} \leq -1.78 \times$ log $K_{oa}+14.56$.

2.2.4.3 Confirmation based on measurements in remote areas

72. The potential for long-range transport of methoxychlor is strongly indicated by monitoring studies and measurements in environmental and biota samples from remote regions.

73. Methoxychlor has been detected in various media in the Arctic, including in air from a region covering the North Pacific to the Arctic Ocean between 2016 and 2017 (concentrations in the range of 0.02–0.42 ng/m^3 (mean 0.08±0.10 ng/ m^3; Gao *et al.*, 2019), in brown snow sampled in May 1988 (0.234 ng/L; Welch *et al.*, 1991), in an ice core drilled in 1998 from an ice cap in the Svalbard archipelago of Arctic Norway (a peak concentration of 4.7 ng/L associated with the early 1980s; Hermanson *et al.*, 2005), in an Arctic lake water (methoxychlor, endosulfan and pentachloroanisole collectively, in concentrations in the range of 0.017–0.023 ng/L; Muir *et al.*, 1995b as cited in AMAP, 1998), in terrestrial, avian and marine biota samples (concentrations in the range of n.d.[15] to 86 ng/g lipid weight (lw) between 1999 and 2005 (Vorkamp *et al.*, 2004 and Savinov *et al.*, 2011; see details in section 2.3 Exposure) and in plants (concentrations in saxifrage were in the range 0.1–1.0 ng/g (dw); France *et al.*, 1998 as cited in AMAP, 1998). Methoxychlor was also detected in seals sampled from the Antarctic Peninsula and South Shetland Islands during the 2013–2014 austral summer (concentrations in the range 7.97–40.13 ng/g lw; Vergara *et al.*, 2019). The presence of methoxychlor at sites remote from known point sources such as the Arctic and Antarctic therefore indicates long-range transport.

74. Monitoring data available from sites remote from known point sources, such as the Arctic and Antarctic demonstrate long-range environmental transport with transfer to the receiving environment, including to biota (Vorkamp *et al.*, 2004; Savinov *et al.*, 2011; Filho *et al.*, 2009; Vergara *et al.*, 2019). In particular, methoxychlor has been found in ice cores in Svalbard (Norway) with peak concentrations between 1979 and 1992 (Hermanson *et al.*,

[15] Not detected (n.d.).

2005 and Ruggirello *et al.,* 2010). The measured levels in ice cores reflect trends in volumes used at lower latitudes, providing further evidence for long-range transport as a source of methoxychlor to these remote regions (see details of the monitoring data in section 2.3.1).

2.2.4.4 Summary of long-range environmental transport

75. While modelling predictions indicate a low potential for long-range transport, monitoring data indicate that transport of methoxychlor to remote areas (Arctic and Antarctic) has taken place. The presence of methoxychlor in remote areas can be explained by atmospheric transport in the gas phase or on particles during dry periods and during periods of lower photolytic activity (Muir *et al.*, 2004; Hoferkamp *et al.*, 2010). Furthermore, considering the collective concentrations of methoxychlor, endosulfan and pentachloroanisole in the range of 0.017–0.023 ng/L in an Arctic lake (Muir *et al.*, 1995b as cited in AMAP, 1998)), and measured levels in surface seawater in the Arctic Ocean and Chukchi Sea (concentrations in the range <MDL–0.38 ng/L (mean 0.15±0.11 ng/L); Gao *et al.*, 2019), long-range transport potential through water and ocean currents is also possible. In the absence of known local or regional sources, detection in environmental and biota samples from the Arctic and Antarctica are resulting from long-range environmental transport of methoxychlor.

2.3 Exposure

2.3.1 Environmental monitoring data

2.3.1.1 Monitoring in remote areas (far from point sources)

76. Methoxychlor has been detected in various media in the Arctic (in air, snow, ice cores, lake waters and marine waters, in biota samples (terrestrial, avian and marine)) and in the Antarctic (in marine biota samples).

77. Hung *et al.* (2005) monitored methoxychlor in air at two Canadian and one Russian Arctic sites (between 1993 and 1995), namely Tagish, Yukon; Kinngait, Nunavut and Dunai Island, Russia. Weekly air samples were collected using a high-volume air sampler that sampled air over 7 days at a time, resulting in approximately 52 samples per year. For each sample, air was drawn through a glass fibre filter (GFF) and two polyurethane foam (PUF) plugs to collect the respective particle and vapour fractions. Field blanks were collected every 4 weeks by handling a PUF and filter in the same manner as a sample, but without airflow, in order to establish method detection limits. Samples of air were taken weekly. The reported air concentration data were not blank- or recovery-corrected. Annual (arithmetic mean) concentrations of methoxychlor were between 0.12 and 0.41 pg/m^3 at the three Arctic sites, representing the sum of methoxychlor collected in the filter and plugs. As Halsall *et al.* (1998) (as cited in Hung *et al.* (2005)) have found, concentrations of organochlorines (OCs) were similar at all sites indicating a uniformity in contamination in Arctic air. Concentrations of methoxychlor in Arctic air were higher than the concentrations found for the POP endrin at the same sites and time (annual arithmetic mean concentrations between 0.15 and 0.29 pg/m^3; before inclusion of endrin to the Stockholm Convention in 2001). Gao *et al.* (2019) monitored methoxychlor in the atmosphere (from 61°N to 31°N) in a region covering the North Pacific to the Arctic Ocean. Samples were collected from 12 July to 23 September 2016 and from 27 July to 7 October 2017. Among three monitored insecticides, methoxychlor was the most abundant insecticide in the atmosphere with concentrations of 0.02–0.42 ng/m^3 (mean 0.08±0.10 ng/ m^3). This contrasts with the undetected results of Su *et al.* (2008) (as cited in Gao *et al.*, 2019) in the Arctic atmosphere. Based on methoxychlor seawater-atmosphere fugacity ratio (between 4.52×10^{-3} and 3.57×10^{-2}), Gao *et al.* (2019) concluded that methoxychlor had higher concentrations in the atmosphere than in seawater.

78. Methoxychlor has also been detected in brown snow in the Canadian Arctic at levels of 0.234 ng/L in May 1988 (Welch *et al.*, 1991), representing the combined concentration of methoxychlor in melted snow and associated filtered particles.

79. Methoxychlor has been detected in the Russian Arctic ice caps at a concentration range of 72–2100 ng/L (MDL of 1 ng/L; Boyd-Boland *et al.*, 1996). However, the date when the samples were collected is not clear. Also, the description of sample preparation is limited, and it is possible that the levels reported represent levels spanning several years or decades (Hoferkamp *et al.*, 2010). A 1998 ice core drilled from the Austfonna ice cap on Svalbard, Norway was analysed for contaminants, with the top 70 m corresponding to the years 1906 (±5) to 1998 (Hermanson *et al.*, 2005). Methoxychlor was found in sections of the core dating to the early 1950s and concentrations were found to increase over subsequent years with a peak concentration of 4.7 ng/L associated with the early 1980s, which is expected to be the period of peak use of methoxychlor globally. The measured levels in the core seem to follow roughly the use volumes at lower latitudes hence providing measured evidence of long-range transport. Ruggirello *et al.* (2010) drilled in April 2005 an ice core on Holtedahlfonna (125 m deep) on Svalbard, Norway to measure the input of pesticides. Methoxychlor was detected in all analysed segments of the ice core beginning 1953–1962. The inputs grew to a peak flux value of 19.6 pg/cm^2/year in the core segment dated 1971–1980 and generally declined from there to the surface layer (10.7 pg/cm^2/year; MDL of 33 pg/L equivalent to ca. 1.7 pg/cm^2/year). The authors compared the ice core burdens and peak flux years in the Holtedahlfonna core (peak flux year

1971–1980) with the ones in the Austfonna core (peak flux year 1986) and noted about a 10 times greater burden in the ice core at Austfonna.

80. Methoxychlor was found in surface seawater in the Arctic Ocean and Chukchi Sea in 2016–2017 (concentrations <MDL–0.38 ng/L (mean 0.15±0.11 ng/L); MDL of 0.01 ng/L; Gao *et al.*, 2019; see further details in section 2.2.2.4 Monitoring data).

81. Measured data in biota are available for methoxychlor at various trophic levels. The substance was found in muscle, liver, blubber or kidney of various terrestrial species (hare, lamb, caribou, muskox), marine invertebrates (snow crab, shrimp, Iceland scallop) and fish (Arctic char, Atlantic cod, Atlantic salmon, wolffish, capelin, shorthorn sculpin), seabirds (thick-billed murre) and marine mammals (ringed seals, harp seals, beluga, minke whale, narwhal) from Greenland in 1998–2001 (Vorkamp *et al.*, 2004), in ringed seals from the Russian Arctic in 2001–2005 (Savinov *et al.*, 2011) and in elephant seals from Antarctica in 1999–2000 (Filho *et al.*, 2009). Methoxychlor concentrations in biota from Greenland were in the range of n.d. to 86 ng/g lw (Vorkamp *et al.*, 2004). The highest concentrations for methoxychlor were observed in the following tissues of species collected from Greenland: snow crab liver (1.7 to 86 ng/g lw), capelin muscle (n.d. to 55 ng/g lw), thick-billed murre muscle (n.d. to 37 ng/g lw), Atlantic cod muscle (n.d. to 33 ng/g lw), shrimp muscle and narwhal liver (n.d. to 32 ng/g lw), muskox blubber (n.d. to 25 ng/g lw), shorthorn sculpin liver (n.d. to 22 ng/g lw), Arctic char muscle (n.d. to 16 ng/g lw), wolfish muscle (1 to 15 ng/g lw) and minke whale muscle (n.d. to 12 ng/g lw)) (Vorkamp *et al.*, 2004). In the Russian Arctic, methoxychlor was detected in the blubber of adult ringed seals from one of three sampling locations (Kalgalaksha Bay, White Sea) at levels ranging from <0.05–8.36 ng/g lw between 2001 and 2005 (MDL of 0.05 ng/g lw; Savinov *et al.*, 2011). Similar levels were found by Vorkamp *et al.* (2004) in the blubber of ringed seals sampled from Ittoqqortoormiit in Greenland (n.d. –7.8 ng/g lw). Methoxychlor was detected at lower levels in the blubber of ringed seals sampled from Qeqertarsuaq in Greenland (n.d. –2.8 ng/g lw; Vorkamp *et al.* (2004)).

82. Methoxychlor has also been measured in elephant seal (*Mirounga leonina*) pups on Elephant Island in Antarctica during the 1999–2000 austral summer (Filho *et al.*, 2009). Mean concentrations of methoxychlor in samples collected from 7 dam/pup pairs of southern elephant seals were: 2.91 ± 1.17 ng/g lipids in dam blubber, 1.79 ± 0.32 ng/g lipids in milk and 1.86 ± 0.40 ng/g lipids in pup blubber. Mean concentrations of methoxychlor in dam/pup blubber and milk were higher than the mean concentrations found for the POPs hexachlorobutadiene (0.38–0.43 ng/g lipids) and α-HCH (0.21–0.39 ng/g lipids) before their inclusion to the Stockholm Convention. The authors concluded that methoxychlor as a contaminant in Antarctica may reflect contamination from potential use at the time of sampling or historical use for agriculture purposes in the southern hemisphere. As these elephant seals are resident to Antarctica (i.e. not a migratory species), the observation of methoxychlor in the tissues of dams and pups suggests long-range transport from sources outside of Antarctica, where uses of these insecticides may still have been occurring during the sampling period of the study (1999–2000).

83. Vergara *et al.* (2019) analysed for methoxychlor in the blubber of adult seals (Southern elephant seals, Antarctic fur seals, Weddell seals, Leopard seals and Crabeater seals) sampled from two locations in the Antarctic Peninsula. Samples were collected during the 2013–2014 austral summer. Mean concentrations of methoxychlor were in the range of 7.97–40.13 ng/g lw (LOD of 1.49 ng/g and LOQ of 4.97 ng/g) in the blubber of all five species of Antarctic seals collected from Gabriel Gonzalez Videal Station. Methoxychlor was detected in the blubber of one of three species collected from Cape Shirreff Field Station, with a mean concentration of 21.92 ng/g lw. The mean concentration of methoxychlor found in Southern elephant seals in this study (27.94 ng/g lw) is higher than the mean concentration found in elephant seals collected from Elephant Island during the study of Filho *et al.* (2009) conducted during the 1999–2000 austral summer (2.91 ± 1.17 ng/g lipids in dam blubber).

84. France *et al.* (1998) (as cited in AMAP, 1998) detected the widely used OCPs, tetra- and pentachlorophenol (as anisoles), endosulfan, methoxychlor, trifluralin, and triallate in lichen and saxifrage from Ellesmere Island (Canada). Concentrations of these pesticides in saxifrage were in the 0.1–1.0 ng/g (dw) range, similar to levels of other individual OCs such as dieldrin and chlordane isomers (before their inclusion to the Stockholm Convention in 2001) (France *et al.*, 1998 as cited in AMAP, 1998).

2.3.1.2 Monitoring in rural and urban areas

85. In 1974, methoxychlor was detected at a concentration of 254 ng/m^3 in air samples collected from a pesticide formulation plant in Southern Florida (USA; WHO (2004) and HSDB (2009)). In a survey (conducted during the years 1987, 1988, and 1989) of pesticide levels in air in two U.S. cities, the mean levels of methoxychlor in indoor, outdoor, and personal air samples from Jacksonville, Florida were 200–300, 0–100, and 100–600 pg/m^3, respectively (EPA 1990e as cited in ATSDR, 2002). Levels of methoxychlor were below the level of detection (approximately 36 pg/m^3) in these air samples in Springfield, Massachusetts. In a survey of ambient air measurements, atmospheric levels of methoxychlor (from data taken at two locations in the United States from 301 samples) ranged from n.d. – 7,000 pg/m^3 (Kelly *et al.*, 1994 as cited in ATSDR, 2002). In Canada, the yearly mean level of methoxychlor in air was 1.7 pg/m^3 from 1988 to 1989 (Hoff *et al.*, 1992 as cited in ATSDR, 2002). Air levels tended to be higher during insect control periods (up to 27 pg/m^3), whereas levels were generally below the detection limit (0.04–0.1 pg/m^3)

during non-use periods (ATSDR, 2002). Sofuoglu *et al.* (2004) collected air samples of OCPs in May 2003 in Izmir (Turkey). Concentrations of methoxychlor in air (reported as the sum on particles and in the gas phase) ranged from 43 to 990 pg/m^3 (mean value: 220±255 pg/m^3; n=20; detection frequency=100%; methoxychlor recovery≥ 110±8).

86. Adu-Kumi *et al.* (2012) measured methoxychlor in urban (mean: 0.8 pg/m^3; n=2) and suburban (range: 0.83–13.25 pg/m^3; mean: 3.88 pg/m^3; n=5) residential air samples from Ghana. Air samples were taken from January to December 2008. Recoveries of surrogate standards were higher than 76% for all samples. Data were not corrected for recovery rate. Guida *et al.* (2018) measured methoxychlor in atmospheric air samples in Brazilian mountains 2000 meters above sea level. Air samples were collected between September 2013 to October 2015 at two National Parks in Southeast Brazil where hundreds of endangered species and many endemic species live. Procedural mean recovery for methoxychlor was 91.08%. Average concentrations of methoxychlor were 9±17 and 19±40 pg/m^3 at the two sites. Minimum and maximum values were in the range of n.d. to 115 pg/m^3 (LOD of 58 pg/m^3). The authors noted that methoxychlor was only measured in two sampling periods at each site, which was less than half of all sampling periods. The authors reported that one of the National Parks was surrounded by intensive agricultural activities which may explain concentration levels found at the sampling site. It should be mentioned that methoxychlor was found in the blank controls (it was found at a concentration above its LOD in only one of them). In addition, the concentrations reported in the samples have been corrected from the blanks of each analytical batch.

87. No data were found for methoxychlor degradation products and their levels in air (ATSDR, 2002).

88. Strachan and Huneault (1979) reported levels of methoxychlor in snow samples collected in February 1976 and in rain samples collected from seven locations in May–November 1976, from the Canadian side of the Great Lakes. Mean concentrations of methoxychlor measured in rain and snow ranged between 1.6–13.1 ng/L (n=50 samples) and 0.1–5.8 ng/L (n=34 samples), respectively. These values were sometimes higher than other measured OCPs such as DDT, lindane and dieldrin, particularly in rain. The authors attributed the presence of methoxychlor in rain in appreciable quantities as a reflection of current use at the time of the study rather than historical use. In a 6-year study (1986–1991) conducted in the Great Lakes Region, the mean annual concentration of methoxychlor in rain was 2.4 ng/L (Chan *et al.*, 1994 as cited in ATSDR, 2002).

89. Only 1 out of 71 groundwater samples from rural areas in the U.S. contained methoxychlor at 0.09 µg/L (or 90 ng/L), but concentrations of methoxychlor up to 50 µg/L (or 50,000 ng/L) were detected in both surface water and groundwater close to agricultural areas where it was applied before its phase out in the U.S. in 2000 (US EPA, 1987). According to Helou *et al.* (2019), measurable levels of methoxychlor (concentration range: n.d. –4.7 ng/L; average: 1.8 ng/L; n=4; sampling in 2011–2012) were detected in grab samples of Litani riverbed surface water (Lebanon). In the same study, methoxychlor was detected below the imit of quantification in grab samples collected from the Orontes River (n=6).

90. Zeng *et al.* (2018) found methoxychlor in the surface water (reservoirs, ponds and stream) of Qingshitan Reservoir in Southwest China from 2014 to 2016. Methoxychlor concentrations in water samples (n=283; 22 sampling sites) ranged from n.d. –13.90 ng/L, with mean values ranging from 2.25 to 2.37 ng/L in reservoirs, ponds and streams and with a detection rates ≥88.57% (the MDLs of OCPs were in the range: 0.02–2.03 ng/L).

91. Affum *et al.* (2018) measured the concentration of pesticides in surface water sources in an agricultural catchment dominated by cocoa crops in the Ankobra River Basin in the Western Region of Ghana. Sampling started a month after the cocoa crops had been sprayed with pesticides and were collected between August and October 2016. Methoxychlor was detected in 54% (n=6) of surface water samples at concentrations in the range of n.d.–0.020 µg/L (or 20 ng/L) (recovery rate: 70%; LOD of 0.010 µg/L or 10 ng/L; Affum personal communication, June 2020). Basheer *et al.* (2002) measured methoxychlor in coastal seawater samples collected in 2002 from the sea surface in the Straits of Johor, located between Singapore and the Malaysian peninsula. Methoxychlor concentrations in seawater were in the range of 0.053–0.616 µg/L or 53–616 ng/L (LOD of 0.041 µg/L or 41 ng/L and methoxychlor recovery ≥92.59±8.14%. The authors mentioned that some minimal agricultural activities remained in Singapore at the time of the measurement.

92. Austria have confirmed that sum parameter measurements of 4,4-methoxychlor (*p,p'*-methoxychlor) and 2,4-methoxychlor (*o,p´*-methoxychlor) were below the limit of detection for lower Austria surface (n=9) or groundwater (n=13) samples collected in 2014 and in 2018. These measurements were made after the ban of its use in Austria in 1993 (Annex E, 2019). Similarly, 4,4-methoxychlor (*p,p'*-methoxychlor) and 2,4-methoxychlor (*o,p´*-methoxychlor) were not detected in 9 of 9 groundwater samples from Austria in 2018. As well, Austria indicated that the sum parameter of methoxychlor (as defined above) was not detected in 15 out of 15 solid waste samples collected in 2016 and in 2018.

93. Methoxychlor has been detected in groundwater at waste disposal sites. A review of groundwater monitoring data from 479 waste disposal sites located throughout the U.S. indicates that methoxychlor was detected in groundwater at 14 (3%) of the sites (Plumb, 1991). According to Helou *et al.* (2019), reported methoxychlor in groundwater sampled in 2012 from the Hasbani basin of Lebanon (average: 0.88 ng/L; range: n.d.–3.4 ng/L; n = 11), and methoxychlor concentrations in the groundwater collected from 15 sites Akkar province in North Lebanon were

in the range of n.d. to 250 ng/L (average: 57 ng/L; n=30; samples collected in 2015). According to Affum *et al.* (2018), methoxychlor was detected in 64% (n=7) of the groundwater samples in the Ankobra Basin (Ghana) at a concentration equal to the LOD of 0.010 µg/L or 10 ng/L (samples collected between August-October 2016; Affum personal communication, June 2020).

94. Abong'o *et al.* (2015) found methoxychlor in soil samples (0–30 cm plough layers) from six farms in the Nyando River catchment area of the Lake Victoria in Kenya. Soil samples were collected over a period of two years in 2005–2006. Of all the measured pesticides, methoxychlor was found in the highest concentration (138.97±1.517 µg/kg soil or 138,970±1,517 ng/kg). The authors state that methoxychlor was commonly used in Kenya at the time of sampling. Bolor *et al.* (2018) found methoxychlor in groundwater and topsoil (0–30 cm depth) samples near farms in Kumasi, Ghana. Samples were collected in September 2014. Methoxychlor was found in the groundwater at 3 of 5 sites and in soil samples collected at all 5 sites at mean concentrations ranging from 1.53–8.87 µg/kg (or 1,530–8,870 ng/kg) and 2.61–58.30 µg/kg (or 2,610–58,300 ng/kg) respectively (LOD of 10 ng/kg and recovery rate: 101.73% (Boadi personal communication, April 2020)). Monitoring data from agricultural soils in Belarus after its ban in 1999, indicate that methoxychlor concentrations in soil samples collected in 2007, 2008 and 2011 were, in most cases, below the detection limit (Annex E, 2019). There is no data regarding the application of methoxychlor to the fields from where the soil samples were collected.

95. Panday *et al.* (2011) measured methoxychlor in surface sediments collected from six different sampling locations along the Yamuna river in Delhi (India). Sediment samples were collected in the pre-monsoon (June), monsoon (August) and post-monsoon (October) seasons in 2006. Methoxychlor was found at all sites for all seasons with concentrations in the range of 7.72–62.78 ng/g. In 2010, another study collected eighty-four samples of surface sediment from 14 locations on either side of the Yamuna river in Delhi (India) during the post-monsoon/winter (February), pre-monsoon/summer (June) and monsoon (September) seasons (Parween *et al.*, 2014). Methoxychlor was detected in sediments only during the monsoon (mean: 0.15 ng/g) and winter (mean: 0.11 ng/g) sampling periods (mean including summer: 0.09 ng/g). Methoxychlor has also been measured in sediments from a coastal lagoon watershed (Argentina) in 2001 (<0.2–127 ng/g; Menone *et al.*, 2001 as cited in Panday *et al.*, 2011), coastal marine sediment (Singapore) in 2005 (<0.4–1.2 ng/g; Wurl and Obbard, 2005 as cited in Panday *et al.*, 2011), from Tampa Bay, Florida (U.S.) in 2004 (0.1 ng/g; Grabe and Barro, 2004 as cited in Panday *et al.*, 2011), and from the Pearl river estuary (China) in 2001 (n.d. –1.49 ng/g; Li *et al.*, 2001 as cited in Panday *et al.*, 2011).

96. Tao *et al.* (2019) analysed OCPs from one sediment core (depth: 20 cm) collected from one bay of the third largest freshwater lake (Lake Taihu) in China in 2012. OCPs recoveries varied from 71.3 to 94.2%. Across the core, which was dated to 1948 to 2012, methoxychlor had the fifth highest average concentration of the 13 OCPs analysed (7.01±2.82 ng/g dry weight (dw); MDL for the OCPs ranged from 0.003 to 0.080 ng/g dw). Castañeda-Chávez *et al.* (2018) measured the concentrations of methoxychlor in sediments (0–20 cm) from 41 sites of the Alvarado lagoon system in Veracruz (Mexico). The sediment collection was performed in triplicate during the dry season corresponding to April–June 2011 (Navarrete-Rodríguez personal communication, January 2020). Concentrations of methoxychlor measured in 20 out of 41 sampling sites were in the range of 1.13–29.40 ng/g dw (mean: 5.650 ng/g dw and standard deviation: 6.561 ng/g dw; LOD for the OCPs of 0.01 ng/g dw).

97. Annex E (2019) information from Canada indicated that methoxychlor was detected in 3 out of the 141 samples of fish collected between 1977 and 1993. Methoxychlor was detected in a single fish sample collected during each of 1981 (2 µg/kg; n = 6), 1988 (18 µg/kg; n = 44), and 1990 (5 µg/kg; n = 9). Fish data show measurable levels of methoxychlor in fish tissues during the period methoxychlor was used in Canada. No fish tissue information is available for Canada after 1993 (Annex E, 2019). Unyimadu *et al.* (2018) found methoxychlor in high concentrations in brackish water fish from the Niger River (Nigeria) (concentration range of 29.3–740.8 µg/kg fresh weight; n=60; recovery rate: 77±3.3 %; fish purchased from the landing sites in July 2009 (Unyimadu personal communication, January 2020)). Enbaia *et al.* (2014) found methoxychlor in fish collected from the Tripoli market in Libya during September-November 2013. Mean concentrations of methoxychlor in 5 of 15 species of fish were in the range of 0.8–4 µg/kg.

98. Nalley *et al.* (1975) measured the concentration of methoxychlor in omental fat samples of raccoons (*Procyon lotor*) live-trapped in two counties of southern Florida. Methoxychlor was detected in 10 of the 20 samples in the concentration range 0.16–3.07 ppm, with a single measurement of 36.82 ppm in one adult female. Salvarani *et al.* (2019) studied concentrations of OCPs in the eggs of two sea turtle species (*Eretmochelys imbricata* and *Chelonia mydas*) collected from the Punta Xen and Isla Aguada (Mexican coast) in 2014 and 2015. Methoxychlor was identified in all 114 of the eggs analysed (mean concentration range of 0.059–1.060 ng/g dw; recovery >85%). According to the International Union for Conservation of Nature (IUCN) Red list, the hawksbill sea turtle (*Eretmochelys imbricata*) is a critically endangered species, while the green turtle (*Chelonia mydas*) is in the category 'least concern'. Buah-Kwofie *et al.* (2018) studied the accumulation of OCPs in fat tissues of live wild Nile crocodiles from iSimangaliso Wetland Park (South Africa). The park forms part of a biodiversity hotspot. A total of 15 crocodiles were sampled in 2016–2017. The sampled population consisted of nine adults and six sub-adults. Methoxychlor concentrations in fat samples of Nile crocodiles were in the range of 79–300 ng/g wet wt (mean value: 170±62 ng/g wet wt; LOD in the range: 0.12–0.4 ng/g wet wt; detection frequency=100%).

99. Cindoruk *et al.* (2020) monitored OCPs in pine (*Pinus pinea*) (needles and branches) from Gemlik (Turkey) between January and December 2016. The maximum concentration determined for methoxychlor was 4.4 ng/g dry weight for pine branches (for OCPs LODs ≤ 3.25 pg and LOQs ≤ 9.087 pg; methoxychlor recovery rate: 69.9 ± 7%; Cindoruk personal communication, May 2020). Methoxychlor had higher concentrations in branches than in needles. Austria indicated that methoxychlor was detected but not quantified (<10 µg/kg) in 1 out of 13 herb samples collected in 2012, after the ban of its use in Austria in 1993 (Annex E, 2019).

2.3.2 Human exposure

100. According to US EPA (2000), the most probable route of exposure to methoxychlor would be from inhalation or dermal contact by workers involved in the manufacture, handling or application of this compound. Farmers and pesticide applicators who use methoxychlor are the populations most likely to receive above average exposures (ATSDR, 2002).

101. The general population can be exposed to low levels of methoxychlor by inhaling dusts and aerosols in air surrounding areas where methoxychlor is used. Based on the results of the Non-Occupational Pesticide Exposure Study (conducted between 1986 and 1988 before its phase out in the U.S. in 2000), inhalation exposure (indoor and outdoor air) to methoxychlor ranged from 0.002 to 0.012 µg/day (for an adult of 70 kg) in Jacksonville, Florida that is representative of an area of U.S. with relatively high pesticide use (US EPA, 1990). In a survey of methoxychlor residues in house dust in 28 homes in an agricultural area of Colorado where pesticide use was common, levels ranged from 1.6 to 103 mg/kg (mean: 14.9 mg/kg) in 8% of the homes of farmers, from 1.9 to 144 mg/kg (mean:18.2 mg/kg) in 9% of the homes of pesticide formulators, and from 1.5 to 29 mg/kg in 2% of the homes of the control group (Starr *et al.*, 1974 as cited in ATSDR, 2002). It is not clear if the presence of methoxychlor in the homes of the control group was due to migration of methoxychlor from nearby buildings or fields where it was applied by farmers, or to in-house use of methoxychlor-containing products by the residents. Populations that live or work on or near a farm where methoxychlor has been used recently on crops or livestock or that live near a hazardous waste site that contains methoxychlor could be exposed to above-average levels of methoxychlor in soil and possibly in water (ATSDR, 2002).

102. Although methoxychlor is poorly soluble in water, it has been found in surface water, groundwater and drinking-water (WHO, 2004).

103. Prior to the phase-out and ban of methoxychlor in the U.S. and Canada, the most likely source of exposure to methoxychlor to the general population outside of higher exposure areas was from low-level contamination of food. In a market basket survey performed in 1980–1982 in 13 American cities, dairy products and cereals/grain products contained levels ranging from a trace to 0.004 mg/kg (Gartrell et al. 1986a, as cited in ATSDR (2002)). The FDA's Total Diet Study program monitors chemical contaminants in the U.S. food supply and has calculated average daily intakes of methoxychlor in adults (age 25–65) ranging from 0.1 to 0.3 ng/kg/day for the period 1986–1991 (Gunderson, 1995b as cited in ATSDR, 2002), 0.6–0.9 ng/kg/day for the period 1984–1986 (Gunderson, 1995a as cited in ATSDR, 2002), and 4 ng/kg/day for the period 1980–1982 (Gartrell et al.,1986b as cited in ATSDR, 2002). A decrease in the average daily intakes of methoxychlor is noted for period 1980–1991. In Canada from 1980 to 1985, methoxychlor was generally not detected in vegetables, fruits, meats, or dairy products (Davies, 1988; Frank *et al.*, 1987b as cited in ATSDR, 2002). However, low levels (4 µg/kg) were detected in strawberries (Frank *et al.*, 1987a as cited in ATSDR, 2002). Exposure to methoxychlor from food may be elevated in persons who consume large amounts of fish and seafood from methoxychlor-contaminated waters. However, fish from the Great Lakes generally did not contain detectable levels of methoxychlor and high levels ranging from 10 to 120 µg/kg were infrequently reported (Camanzo *et al.*, 1987; Devault, 1985 as cited in ATSDR, 2002).

104. The 2018 pesticide monitoring results in the EU (including Iceland and Norway) (EFSA, 2020) summarised the results provided by the reporting countries and identified areas of concern regarding sample compliance with the legal limits of pesticide residue in foods. EFSA also assessed the consumer dietary exposure to pesticide residues in the sampled food commodities and performed an analysis of the chronic and acute dietary risks for European consumers. Based on the analysis of the 2018 pesticide monitoring results in the EU (including Iceland and Norway) (EFSA, 2020), methoxychlor was quantified in 5 out of 56,428 food samples from 30 countries (quantification rate 0.01%). Methoxychlor was quantified in one animal product sample (in equine fat) and in four coffee bean samples (imported from outside the EU: Brazil, Ethiopa, Peru and Uganda) at concentrations in the range of 0.01-0.05 mg/kg (LOQ of 0.01 mg/kg). It was concluded that the acute dietary exposure to this pesticide, would not be expected to pose a concern to consumer health. The chronic exposure to methoxychlor is according to the lower and adjusted upper bound scenarios in the range of 0.095-1.9 % of the ADI. EFSA concluded that the chronic dietary exposure to methoxychlor residues in food commodities analysed, is unlikely to pose concerns for consumer health. The outcome of the pesticide monitoring for 2013 and 2016 is presented in the Appendix.

105. Shaker and Elsharkawy (2015) analysed raw buffalo milk samples from Upper Egypt for OCPs. Samples were collected in the city of Assiut between February and August 2013. According to the authors, pesticides in milk originate from contaminated feed, grass or corn silage, and from direct application of pesticides on dairy cattle. Methoxychlor was detected in 66% of the samples (n=30). Concentrations of methoxychlor in raw buffalo milk were

in the range of 0.130–0.200 mg/kg. Methoxychlor was banned in Egypt in 1996, however, according to the drafter of this dossier, the levels detected in buffalo milk suggest the possibility that it was still being used in Egypt at the time of sampling.

106. Bolor *et al.* (2018) studied the levels of OCP residues in 3 different vegetables: lettuce, onion and cabbage from farms in Kumasi, Ghana in September 2014. Mean concentrations of methoxychlor in the vegetables were in the range of 9.02–184.1 µg/kg (or 0.009–0.184 mg/kg). The authors reported that the measured concentrations were higher than the EU MRL of 0.01 mg/kg for these vegetables (EU Pesticides Database, 2019). However, rather than being based on specific risks, the EU MRL for methoxychlor is based on the default lowest limit of analytical determination in EU law. Furthermore, Bolor *et al.* (2018) reported that estimated daily intakes of methoxychlor resulting from the levels measured in vegetables did not exceed an acceptable daily intake (ADI) of 0.005 mg/kg/day established by the US EPA. Therefore, the levels reported did not result in identified risks to the population. However, levels detected in some of the vegetables significantly exceeded levels in corresponding soil samples.

107. Adeleye *et al.* (2019) studied concentration of methoxychlor in leafy vegetables (amaranth and fluted pumpkin) collected in November 2017 to January 2018 in southwestern Nigeria. The mean concentration of methoxychlor in amaranth was 4.590±2.774 mg/kg dw and 6.223±2.489 mg/kg dw in fluted pumpkin (LOD of 0.0091 mg/kg and recovery rate of 96.19%). The authors estimated the non-carcinogenic health risk (based on the reference dose for reproductive and developmental effects (RfD) of 0.005 mg/kg/day) for amaranth and fluted pumpkin and found that the hazard quotient for methoxychlor was above 1 for both children and adults thus representing a potential risk for consumers for these vegetables. The authors reported that the use of water from the river by farmers might contribute to the OCP residues detected in the vegetables. According to Adeleye *et al.* (2019), contamination can come from the soil on which the vegetables were planted or from long-range transportation of the OCPs applied to crops elsewhere.

108. Exposure was monitored in two non-occupational populations that live in areas where farming was extensive. Methoxychlor in blood serum was studied in 39 men living in Southern Honduras (Steinberg *et al.*, 1989 as cited in ATSDR, 2002). Serum samples were collected in 1988. Of the population studied, 20 men, representing the test group, lived and worked in and near three farming cooperatives near Choluteca where pesticide use is extensive (16–30 times/year), and 19 of the men, representing a comparison group, lived in a town near Choluteca where pesticides are applied only once per season. Methoxychlor was detected in the serum of one man of the comparison group at 5.16 mg/L, but none was detected at or above the detection limit (<0.24–4.07 mg/L) in the remaining participants. However, Carreño *et al.* (2007) found levels of OCPs in the serum of 220 healthy young men (mean age: 20.75 years old; range: 18–23 years old) living in areas Southern Spain characterized by extensive greenhouse agriculture. The sampling date is unknown. Mean concentration of methoxychlor in the serum samples of young men was 2.84 ng/mL (standard deviation: 5.09 ng/mL; maximum: 53.8 ng/mL; n=220; frequency: 60.7%; recovery rate for methoxychlor is unknown).

109. There are little data about children's exposure to methoxychlor or its degradation products. A child's exposure may differ from an adult's exposure in many ways. Children are especially vulnerable to pesticides exposure because they consume a larger amount of food and water relative to their body weight as compared to adults. Small children may play close to the ground and are therefore more likely than adults to come in contact with dirt (dermal exposure) and dust found in home carpets, dirt found outside the home, and lawns (inhalation of airborne soil particles). Children also may intentionally or unintentionally ingest soil that contains low levels of methoxychlor (ATSDR, 2002).

110. In a study conducted in residential areas of North Carolina (the Research Triangle area), house dust was collected in November 1996 (prior to the ban of methoxychlor in the U.S.), separated into seven size fractions (<4–500 µm in diameter), and monitored for pesticide residues (Lewis *et al.*, 1999 as cited in ATSDR, 2002). The concentration of methoxychlor reported by the authors for various dust particle sizes is as follows: 250–500 µm, 120 ng/g; 150–250 µm, 210 ng/g; 106–150 µm, 310 ng/g; 53–106 µm, 570 ng/g; 25–53 µm, 740 ng/g; 4–25 µm, 680 ng/g; and <4 µm, 1,000 ng/g. Lewis *et al.* (1999; as cited in ATSDR, 2002) state that ingestion, dermal exposure, and inhalation of house dust may represent a substantial portion of a child's exposure to pesticides. In another study, foods that are representative of the diets of eight population groups (ranging from infants to elderly adults) were prepared for consumption and analyzed for pesticide residues using the methods in the FDA's revised (April 1982) Total Diet Study (Gunderson, 1995a as cited in ATSDR, 2002). The mean daily intakes for children and young adults for the test period 1986–1991 were calculated to be 0.4 ng/kg/day for 6–11-month old infants, 0.9 ng/kg/day for 2-year-old children; 0.3 ng/kg/day for 14–16-year-old females and 0.4 ng/kg/day for 14–16-year-old males (Gunderson, 1995b as cited in ATSDR, 2002). Intakes of methoxychlor for the test period 1984–1986 were calculated to be 1.0 ng/kg/day for 6–11-month-old infants; 2.4 ng/kg/day for 2-year-old children; 0.8 ng/kg/day for 14–16-year-old females; 0.6 ng/kg/day for 14–16-year-old males; and 0.6–0.9 ng/kg/day for 25–65-year-old adults (Gunderson, 1995a as cited in ATSDR, 2002). Intakes of methoxychlor for the test period 1980–1982 were calculated to be 19 ng/kg/day for 6–11-month-old infants, 4 ng/kg/day for 2-year-old toddlers, and 4 ng/kg/day for 25–65-year-old adults (Gartrell *et al.*, 1986b as cited in ATSDR, 2002). A reduction in the estimated daily intake of methoxychlor is noted for the period 1980–1991 for all population groups.

111. Other possible sources of concern for exposure of methoxychlor to children are parents' work clothes and equipment used to apply products that contain methoxychlor. This mode of exposure would be of special concern in agricultural areas and around homes where pesticides are applied for lawn and garden or indoor use (ATSDR, 2002). Lopez-Espinosa *et al.* (2008) investigated the presence of OCP residues in 52 fat samples of boys (mean age 7 years old (0–15 years old)) living in Southern Spain. The occupations and activities of the parents of the boys are unknown; however, the samples do provide information about the exposure to children in a predominantly agricultural area. According to the authors, children can be exposed to OCPs *in utero* via the placenta (Lopez-Espinosa *et al.*, 2007) and after birth via lactation (Noren and Meironyte, 2000; Solomon and Weiss, 2002). Fat samples were collected between 1994 and 1996, while methoxychlor was still in use in the EU. Methoxychlor was found in adipose tissues of 3 out of 52 children with a mean concentration of 16±20 ng/g lipid (Percentiles among ≥ LOD: P25=52 ng/g lipid; P50=121 ng/g lipid and P75=680 ng/g lipid). The mean concentration (±S.D.) was calculated for the whole group and 25, 50, and 75 percentiles were calculated for samples with methoxychlor concentrations ≥LOD. Raw data are not available and it is unclear how data <LOD have been considered in the calculation of the mean concentration and if on which distribution was used for calculating the percentiles.

112. In animals, methoxychlor and metabolites of methoxychlor that are estrogenic can be transferred from a nursing mother to her newborn babies through breast milk. Methoxychlor and its metabolites can probably cross the placenta and have been detected in human breast milk (ATSDR, 2002).

113. Damgaard *et al.* (2006) studied 62 milk samples from mothers of cryptorchid boys (29 Danish and 33 Finnish) and 68 from mothers of healthy boys (36 Danish and 32 Finnish). This study was performed in Finland and Denmark from 1997 to 2001, while methoxychlor was still in use in the EU. Concentrations of methoxychlor in human breast milk samples were in the range of 0.06–1.12 ng/g lipid (milk of mothers of boys with cryptorchidism) and 0.05–0.41 ng/g lipid (milk of mothers with healthy boys) with a total detection rate of 26.9%. There were no significant differences in the most common confounders (lipid content in breast milk, maternal age, maternal BMI, parity and smoking habits, gestational age, birth weight and length of the boys) between the case and the control groups of the study, with the exception of the Danish group where gestational age differed slightly. Methoxychlor was measured at slightly higher concentrations in milk from mothers giving birth to cryptorchid boys than in mothers giving birth to healthy boys. However, there were no statistically significant differences between cryptorchid and healthy boys for methoxychlor (Damgaard *et al.*, 2006). Kao *et al.* (2019) found methoxychlor in breast milk of 26 out of 55 (detection rate 47%) healthy mothers (mean age 29.5 years old) in southern Taiwan Province of China. Milk samples were collected between 2007 and 2010. Mean concentration of methoxychlor in breast milk was 0.106±0.149 ng/g lipid (range values: n.d. –0.620 ng/g lipid; n=55; n>LOD = 26; LODs for the OCPs were in the range: 0.0151 to 0.0540 ng/g lipid). According to the authors, OCPs have been banned in Taiwan Province of China, since 1975.

114. Luo *et al.* (2016) measured the level of OCPs in the umbilical cord blood from 972 healthy pregnant women (mean age: 26.6 years old) residing in the Huaihe River Basin (China). None of the women had been occupationally exposed to OCPs. The recoveries of all compounds ranged from 72–119% in plasma samples and LODs and LOQs were 0.08–2.31 ng/mL and 0.26–7.69 ng/mL, respectively. Samples were collected between November 2013 and June 2014. Methoxychlor was one of the OCPs most frequently found in samples (range: n.d.–33.75 ng/mL; mean: 0.98±1.42 ng/mL, median 1.00 ng/mL, frequency: 74.49%; recovery rate: 98.5%). Concentrations of methoxychlor in cord blood were significantly and positively correlated with consumption of red meat (including pork, beef and lamb) of mothers from the study. The authors compared the levels they found in umbilical cord bloods with levels found in other countries or regions. They reported that the levels of methoxychlor in umbilical cord bloods were higher in their study than that of Shanghai, China (mean level: 0.0259 µg/L or 25.9 ng/L, median 0.02 µg/L or 20 ng/L, maximum 1.23 µg/L or 1,230 ng/L, frequency: 1.5% in samples collected in 2008–2009 from 1438 pregnant women; Cao *et al.*, 2011 as cited in Luo *et al.* (2016)) and lower than that of Granada, Spain (mean level 3.32±4.01 ng/mL, median 1.44 ng/mL; maximum: 21.51 ng/mL; frequency: 34.4%; samples were collected in 2000–2002 in a cohort of 318 mother-son pairs (mothers mean age: 31.91 years old); Mariscal-Arcas *et al.*, 2010 as cited in Luo *et al.*, 2016).

115. Cabrera-Rodriguez *et al.* (2020) found methoxychlor in 4 out of 447 (0.9%) umbilical cord blood samples from La Palma (Canary Islands, Spain) collected between March 2015 and April 2016 with laboratory recoveries ≥ 98.2% for methoxychlor (Pérez Luzardo personal communication, April 2020). Concentrations of methoxychlor in umbilical cord blood samples were in the range 0.004–0.132 ng/mL, while methoxychlor was banned in the EU in 2006. Jimenez Torres *et al.* (2006) determined the level of OCPs in the adipose tissue and serum of 72 women giving birth (aged from 18–35 years) in Southern Spain. The date of sampling is unknown. Methoxychlor was found in 3 out of 72 (4.1%) adipose tissue samples from pregnant women at a concentration range of 106–817.85 ng/g of fat (mean value: 347.73 ng/g of fat; SD: 407.19 ng/g of fat; recovery rate of 97%). However, methoxychlor was not detected in the serum.

2.4 Hazard assessment for endpoints of concern

116. No harmonised classification is reported for methoxychlor at the EU level (cf. Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation)) nor elsewhere. EU notified classifications and labellings are reported in the Appendix. According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life (with long lasting effects), may cause damage to organs, is harmful (if swallowed, in contact with skin, if inhaled) and is suspected of damaging fertility or the unborn child.

2.4.1 Ecotoxicological effects

2.4.1.1 Adverse effects on aquatic organisms

117. US EPA (2004) determined that, based on available data, methoxychlor is classified as very highly toxic ($LC50$[16] < 0.1 mg/L) to aquatic animals (fish and invertebrates) on an acute exposure basis. Toxicity estimates were as low as 0.0005 mg/L for freshwater invertebrates (US EPA, 2004). Invertebrates species appear to be the most sensitive species in the available acute and chronic tests for methoxychlor. From Anderson and DeFoe (1980), HSDB (2009) and US EPA (2019), acute lethal concentrations (LC50s) for aquatic invertebrates were mostly below 1 µg/L or 1,000 ng/L and around 10 µg/L or 10,000 ng/L for fish. Chronic NOECs are scarce for methoxychlor but those reported for fish and aquatic invertebrates are below 1.3 µg/L or 1,300 ng/L.

118. The conclusions presented in the previous paragraph are confirmed by the study made by Maltby *et al.* (2005). The authors calculated the species sensitivity distribution (SSD) for methoxychlor based on acute toxicity data (due to the lack of NOEC values for this substance) and a log-normal model. SSDs are used to calculate the concentration at which a specified proportion of species will be affected, referred to as the hazardous concentration (HC) for *p* (%) of species (HC*p*). The most frequently estimated HCs are the HC_5. Data used in the SSD were not provided in the reference but were reported to have been collated from existing toxicity databases (e.g., RIVM database), published literature, and unpublished industry data. Selected endpoints were median lethal concentration or median effect concentration (EC_{50}) regarding immobility for animals. Test duration was 2 to 21 days for fish and 1 to 7 days for invertebrates. HC_5 estimates for methoxychlor, calculated using the ETX software were 0.47 µg/L or 470 ng/L for aquatic arthropods (0.37 µg/L or 370 ng/L when restricting to freshwater arthropods) and 4.56 µg/L or 4,560 ng/L for aquatic vertebrates. It was concluded that methoxychlor is more toxic to arthropods than vertebrates (fish and amphibians). In addition, methoxychlor appears to have similar toxicity to freshwater arthropods, aquatic arthropods and vertebrates as the POP lindane (HC_5 of 1.7 µg/L or 1,700 ng/L for freshwater arthropods, HC_5 of 0.79 µg/L or 790 ng/L for aquatic arthropods and HC_5 of 4.84 µg/L or 4,840 ng/L for vertebrates).

119. Whether methoxychlor causes estrogenic effects in aquatic wildlife has been investigated in a number of experiments (ATSDR, 2002). Data indicate that methoxychlor has endocrine disruptive effects in fish (Smeets *et al.*, 1999 as cited in ATSDR, 2002), amphibian (Ingermann *et al.,* 1997 and Ingermann *et al.,* 1999 as cited in ATSDR, 2002), and sea urchin (Mwatibo and Green, 1997 as cited in ATSDR, 2002) fertility, growth, and development. Sometimes the effects of methoxychlor parallel those of estradiol and sometimes there are either subtle or drastic differences from the effects of estradiol. Thus, it is likely that not all of the effects of methoxychlor in aquatic wildlife are mediated through estrogen receptors. There may be many different mechanisms involved in the varied effects and the different species (ATSDR, 2002). Methoxychlor was reported to exhibit thyroid activity in the amphibian metamorphosis assay (AMA) but not in any rodent screens, but the range of chemicals tested in the former is less than in the latter (Pickford (2010) and OECD (2012b)).

120. No ecotoxicity data have been found for benthic sediment-dwelling organisms.

2.4.1.2 Adverse effects on terrestrial organisms

121. According to US EPA (2004), methoxychlor is practically nontoxic to birds on both an acute oral (LD_{50} >2000 mg/kg) and a subacute dietary (LC_{50} > 5000 mg/kg/day) exposure basis. However, US EPA (2004) mentioned that methoxychlor's effects on the hypothalmic-pituitary-gonadal axis along with the developmental effects raise concern for the potential endocrine disrupting effects of methoxychlor on non-target animals. These concerns are underscored by the structural similarity of methoxychlor to DDT. The lack of avian reproduction data on methoxychlor is a major uncertainty given the recognized chronic reproductive risk associated with DDT through thinning eggshells.

122. According to US EPA OPP Pesticide Ecotoxicity Database, methoxychlor has a LD_{50}-48h of 23.57 µg/bee (contact LD_{50} studies) for *Apis mellifera* (Honey bee). Technical grade methoxychlor and adult bees were used for testing. This study has been considered as core (acceptable) data by US EPA. The test was performed following

[16] Acute toxicity related to waterborne exposure is generally expressed in terms of a concentration which is lethal to 50% of the test organisms (lethal concentration, LC_{50}), causes a measurable adverse effect to 50% of the test organisms (e.g. immobilization of daphnids), or leads to a 50% reduction in test (treated) organism responses from control (untreated) organism responses (e.g. growth rate in algae) following an exposure in the range of hours to days, expressed as effective concentration, EC_{50}.

guideline: [141-1] Honey Bee Acute Contact LD_{50} (FIFRA 158.590). This test was an acute contact study: methoxychlor was topically applied or dermally adsorbed (US EPA, 2019).

123. According to ATSDR (2002), oral exposure of laboratory animals to methoxychlor has shown that high doses of methoxychlor are capable of causing neurological injury (tremors, convulsions etc, observed at large acute doses of ≥ 1,000 mg/kg/day methoxychlor orally administered to rats by gavage in oil (LOAEL[17] of 2,500 mg/kg/day (decreased locomotor activity) and 3,000 mg/kg/day (tremors); Cannon Laboratories, 1976 and Dikshith *et al.*, 1990 as cited in ATSDR, 2002 and 1,000–4,000 mg/kg/day methoxychlor administered to dogs for 8–24 weeks; Tegeris *et al.*, 1966 as cited in ATSDR, 2002), but most studies indicate that the reproductive system is the most sensitive target for methoxychlor. The resultant types of reproductive effects are indicative of interference with the normal actions of estrogen or androgen. According to ATSDR (2002), mechanistic studies have confirmed that metabolites of methoxychlor can compete with estrogen for binding to estrogen receptors and can mimic some and antagonize other effects of estrogen. Additional studies have shown that methoxychlor or its metabolites can interact with the androgen receptor and antagonize androgenic effects (ATSDR, 2002). These actions are further supported by predictions from the ToxCast models (US EPA, 2020b). Methoxychlor is predicted to be a weak estrogen agonist (weak estrogen binder), possibly a weak estrogen antagonist too, as well as an androgen antagonist. In females, these interactions can result in disruption of estrus cyclicity (precocious vaginal opening and estrus observed in female rats exposed *in utero*, during lactation and postweaning to 5–150 mg/kg/day (LOAEL of 5 mg/kg/day for precocious vaginal opening); Chapin *et al.*, 1997 and Harris *et al.*, 1974 as cited in ATSDR, 2002), reduced fertility (observed in female rats following intermediate-duration oral exposure to 50–150 mg/kg/day methoxychlor between gestation day 14 and postnatal day 42 (LOAEL of 50 mg/kg/day); Chapin *et al.*, 1997 as cited in ATSDR, 2002), and increased pre- and post-implantation losses (in female rats following acute oral exposure to 100–250 mg/kg/day laboratory grade methoxychlor (NOAEL[18] of 100 mg/kg/day and LOAEL of 250 mg/kg/day); Cummings and Gray, 1987, 1989; Cummings and Laskey, 1993; Cummings and Perreault, 1990 as cited in ATSDR, 2002).

124. Effects in males can include delayed sexual maturation (observed in male rats orally exposed to 100–400 mg/kg/day methoxychlor beginning on postpartum day 21 for 56 days to 10 months (LOAEL of 100 mg/kg/day); Gray *et al.*, 1989, 1999 as cited in ATSDR, 2002), atrophy of reproductive organs and accessory glands (observed in male rats orally exposed to 50–150 mg/kg/day methoxychlor; Chapin *et al.*, 1997 as cited in ATSDR, 2002), and altered sexual or socio-sexual behaviour (observed in male rats exposed *in utero*, during lactation and postweaning to 60 mg/kg/day (LOAEL) for 6 weeks; Harris *et al.*, 1974 as cited in ATSDR, 2002). Many of these effects may be mediated through altered hormone levels (ATSDR, 2002). Some of these effects were also reported by Aoyama *et al.* (2012).

125. Aoyama *et al.* (2012) conducted a two-generation reproduction toxicity study in rats in accordance with OECD TG 416. Both sexes of SPF Sprague Dawley rats were exposed to methoxychlor in their diet (0, 10, 500 and 1500 ppm) for two successive generations. In females, the following reproductive effects were observed at 500 and 1500 ppm: prolonged estrous cycle, reduced fertility, decreased numbers of implantation sites and fewer newborns, decreased ovary weights and/or increased incidences of ovarian cysts. Estrogenic effects were demonstrated by an increase in uterine weights of weanlings at 500 and 1500 ppm. The following adverse effects on the male reproductive system were observed: delayed preputial separation (F1), and decreased testicular spermatid head and/or epididymal sperm counts with significant reductions in weights of testes, seminal vesicles (with atrophy), epididymal, and prostate weights (with atrophy) (Parent and F1). The authors concluded that the NOAEL was a dose level of 10 ppm (at least 0.600 mg/kg per day for males and 0.866 mg/kg per day for females).

126. Observable changes in the liver (increased relative liver weight (observed in rats orally administered lethal doses of 500–1,200 mg/kg/day methoxychlor for 13–16 weeks (NOAEL of 90 mg/kg/day and LOAEL of 1,200 mg/kg/day); Davidson and Cox, 1976 and Dikshith *et al.*, 1990 as cited in ATSDR), altered enzyme and protein levels (observed in rats that received 100–1,000 mg/kg/day methoxychlor for 90 days; Dikshith *et al.*, 1990 as cited in ATSDR, 2002), pale and mottled appearance (observed in pregnant rabbits orally administered 35.5 mg/kg/day (LOAEL) methoxychlor on days 7–19 of gestation (NOAEL of 5 mg/kg/day); Kincaid Enterprises, 1986 as cited in ATSDR, 2002) and kidneys (cystic tubular nephropathy (observed in rats orally exposed to 861 mg/kg/day methoxychlor in the feed for 35–55 days; Tullner and Edgcomb, 1962 as cited in ATSDR, 2002), elevated blood urea nitrogen [BUN]) of animals (observed in pigs orally exposed to 1,000 mg/kg/day methoxychlor in the feed for 24 weeks; Tegeris *et al.*, 1966 as cited in ATSDR, 2002)), as well as weight loss, are caused only by relatively large doses of methoxychlor; these effects are probably not mediated by an estrogenic mechanism (ATSDR, 2002).

127. In animals, signs of fetotoxicity (decreased fetal body weight (observed in pups after pregnant rats were orally exposed to 200 mg/kg/day (LOAEL) on days 6–15 of gestation; Khera *et al.*, 1978 as cited in ATSDR, 2002), increased incidence of wavy ribs (observed in rats orally exposed to 40.8 mg/kg/day (LOAEL) or more methoxychlor on days 6–15 of gestation; Culik and Kaplan, 1976 as cited in ATSDR, 2002), resorptions of fetuses (observed in rats and rabbits exposed to 17.8–400 mg/kg/day; Culik and Kaplan 1976, Khera *et al.*, 1978 and Kincaid Enterprises, 1986

[17] Lowest Observed Adverse Effect Level (LOAEL).

[18] No Observed Adverse Effect Level (NOAEL).

as cited in ATSDR, 2002), and death were noted following exposure to methoxychlor *in utero*. These effects occurred in the presence of maternal toxicity and may not be true signs of treatment-related developmental toxicity (ATSDR, 2002).

128. Methoxychlor exposure during development can adversely affect the reproductive system of rats and mice in both developing and adult animals. These effects are the result of the disruption by estrogenic methoxychlor metabolites of the normal delicate balance of time-sensitive hormone levels during fetal and post-natal development (ATSDR, 2002). Further information on methoxychlor effects during developmental exposure such as reduced ovulation and fertility and premature aging, possibly by altering ovarian gene expression and folliculogenesis can be found in methoxychlor proposal (UNEP/POPS/POPRC.15/4).

129. Manikkam *et al.* (2014) examined the actions of methoxychlor to promote the epigenetic transgenerational inheritance of adult-onset disease and associated differential DNA methylation regions (i.e. epimutations) in sperm. Gestating F0 generation female rats were transiently exposed (200 mg/kg bw/day (4% of oral LD_{50}) via intraperitoneal injection in DMSO) to methoxychlor during fetal gonadal development (gestation days 8 to 14). Adult-onset disease was then evaluated in adult F1 and F3 generation progeny for control (vehicle exposed) and methoxychlor lineage offspring. There were increases in the incidence of kidney disease, ovary disease, and obesity in the methoxychlor lineage animals. In females and males, the incidence of disease increased in both the F1 (kidney weights increased in males and females, testis weight decreased, ovarian disease increased (primordial follicle pool decrease) and the F3 generations (kidney disease increased in females, ovarian disease increased (presence of ovarian cysts), obesity in females increased) and the incidence of multiple disease increased in the F3 generation (kidney disease, ovarian disease and obesity).

130. Furthermore, Manikkam *et al.* (2014) observed increased disease incidence in F4 generation rat reverse outcross (female) offspring indicating disease transmission was primarily transmitted through the female germline. Analysis of the F3 generation sperm epigenome of the methoxychlor lineage males rats identified differentially DNA methylated regions (DMR) termed epimutations in a genome-wide gene promoters analysis. These epimutations were found to be methoxychlor exposure specific in comparison with other exposure specific sperm epimutation signatures. Observations indicate that methoxychlor has the potential to promote the epigenetic transgenerational inheritance of disease and the sperm epimutations appear to provide exposure specific epigenetic biomarkers for transgenerational disease and ancestral environmental exposures. Similar epigenetic transgenerational effects on male fertility were found by Anway *et al.* (2005).

131. No ecotoxicity data have been found for soil organisms while this compartment is considered as the most relevant compartment due to the use of the substance.

2.4.1.3 Summary of ecotoxicological effects

132. Based on available data, methoxychlor has been classified as very highly toxic to aquatic invertebrates and fish (US EPA, 2004). Aquatic arthropods are more acutely sensitive species than fish, with a HC_5 of 0.47 µg/L or 470 ng/L for aquatic arthropods (0.37 µg/L or 370 ng/L when restricting to freshwater arthropods). Methoxychlor is suspected to have endocrine disruptive effects in fish, amphibian, and sea urchin fertility, growth, and development. Methoxychlor is practically acutely or subacutely nontoxic to birds, but reproductive toxicity has not been tested. In view of the structural similarity of methoxychlor to DDT, the lack of avian reproduction data on methoxychlor is a major uncertainty given the recognised chronic reproductive risk associated with DDT through thinning eggshells. Animal studies indicate that high doses of methoxychlor are capable of causing neurological injury (tremors, convulsions), but most studies indicate that the reproductive system is the most sensitive target for methoxychlor. The resultant types of reproductive effects are indicative of interference with the normal actions of estrogen or androgen. Furthermore, observations in rats indicate that methoxychlor has the potential to promote the epigenetic transgenerational inheritance of disease and associated sperm epimutations.

2.4.2 Adverse effects on human health

133. Based on a review of all the available data, IARC has classified methoxychlor as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) (IARC, 1987). Similarly, EPA has classified methoxychlor as a Group D carcinogen (not classifiable as to human carcinogenicity on the basis that human data are unavailable and animal evidence is inconclusive) (ATSDR, 2002 and IRIS, 2003). However, a study by Kim *et al.* (2014) demonstrated that methoxychlor may induce ovarian cancer cell growth by distinctly disrupting cyclin D1, p21 and Bax expressions in ER-positive BG-1 ovarian cancer cells. Methoxychlor is likely to be a tumour promoter because it disturbs the metabolic cooperation between 6-thioguanidine-sensitive and -resistant V79-cells (WHO, 2004). In addition, a weakly positive increase was observed in a transformation study using BALB/3T3 cell line (Dunkel *et al.*, 1981 as cited in IRIS, 2003).

134. The genotoxic potential of methoxychlor appears to be negligible (WHO, 2004). In mutagenicity assays, negative results were obtained (with or without metabolic activation) in bacteria, yeast, in assays of methoxychlor-induced DNA damage, or in assays of unscheduled DNA synthesis in mammalian cell cultures (Probst *et al.*, 1981 as

cited in IRIS, 2003). When testing mutagenicity in humans, methoxychlor did not produce mutations at the thymidine kinase (TK) locus in human lymphoma cells (Caspary *et al.*, 1988 as cited in ATSDR, 2002).

135. Although human data on the reproductive effects of methoxychlor are limited, the animal and *in vitro* data strongly suggest that sufficient exposure to methoxychlor may adversely affect the development, histopathology, and function of the human reproductive system (ATSDR, 2002). There are no epidemiological studies concerning adverse effects on the reproductive system following exposure to methoxychlor, but *in vitro* studies reveal that human liver microsomes are capable of metabolising methoxychlor to metabolites with higher estrogenic activity (ATSDR, 2002).

136. Methoxychlor exposure during development can adversely affect the reproductive system of both developing and adult animals. These effects are the result of the disruption by estrogenic methoxychlor metabolites of the normal delicate balance of time-sensitive hormone levels during fetal and post-natal development. Taken together, the animal data suggest that human exposure to methoxychlor during critical stages of development may adversely affect reproductive development, causing effects that may not be detected until after puberty (ATSDR, 2002).

137. According to US EPA (2000), an OSHA PEL of 15 mg/m^3 has been derived for methoxychlor (Occupational Safety and Health Administration's permissible exposure limit expressed as a time weighted average; the concentration of a substance to which most workers can be exposed without adverse effect averaged over a normal 8-hour workday or a 40-hour work-week).

138. A minimal risk level is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. A minimal risk level of 0.005 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to methoxychlor. This minimal risk level is based on a LOAEL of 5 mg/kg/day (lowest dose tested) from gestation day 14 to postpartum day 42 for accelerated onset of puberty (i.e., precocious vaginal opening) in immature female rats exposed to methoxychlor *in utero*, during lactation, and after weaning. Precocious vaginal opening was evident (statistically significant) in all methoxychlor-treated groups (vaginal opening occurred on postnatal days 37.4, 35.2, 30.8, and 33.4, respectively, for groups 0, 5, 50, and 150 mg/kg/day). The LOAEL was divided by an uncertainty factor of 1,000 (10 for variation in sensitivity among humans, 10 for extrapolation of animal data to humans, and 10 for extrapolation from a LOAEL to a NOAEL) (ATSDR, 2002).

139. In general, the reference dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (ATSDR, 2002). An RfD of 0.005 mg/kg/day (for chronic oral exposure) was derived (in 1990) by the US EPA based on the No Observed Effect Level (NOEL) of 5.01 mg/kg/day for maternal toxicity (observed as excessive loss of litters (abortions) in the mid- (35.5 mg/kg/day) and high- dose groups (251.0 mg/kg/day)) in rabbits dosed during gestational days 7–19 (IRIS 2003; Kincaid Enterprises 1986 as cited in ATSDR, 2002). However, these values were based on an incomplete database, as several regulators (US EPA, 2004) noted that deficiencies in the available data precluded a comprehensive risk assessment. Accordingly, the NOEL was divided by an uncertainty factor of 1,000 (100 for the inter-and intraspecies differences and 10 for the poor quality of the critical study and for the incompleteness of the database on chronic toxicity).

140. A tolerable daily intake (TDI) is an estimate of the amount of a substance in air, food or drinking water that can be taken daily over a lifetime without appreciable health risk. A TDI value of 0.005 mg/kg/day for methoxychlor was derived by WHO (2004) based on the NOEL of 5.01 mg/kg/day for maternal toxicity in rabbits. Allocation of 10% of the TDI to drinking-water results in a guideline value of 20 µg/L (WHO, 2004).

2.4.2.1 Epidemiological studies

141. An epidemiological study of men in Minnesota and Iowa suggested an association between leukemia and farming (Brown *et al.*, 1990). In this study, there was an increased odds ratio (OR) of 2.2 for methoxychlor. This represented 11 cases of leukemia among 578 farmers with occupational exposure to methoxychlor versus 16 cases out of 1,245 controls with no known exposure. The OR of 2.2 for methoxychlor followed adjustments for confounders including vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations and high-risk exposures. This study suggests a possible association between methoxychlor exposure and leukemia. However, a definitive conclusion on the relationship between methoxychlor exposure and leukemia is not possible based on this single study with exposure to multiple pesticides and multiple risk factors.

142. Mills and Yang (2006) evaluated breast cancer incidence rates from the California (USA) Cancer Registry (CCR) that includes state-wide and county-and race/ethnic-specific data from 1988–1999. The authors used regression analysis to evaluate breast cancer incidence as a function of organochlorine use in California after controlling for known risk factors for breast cancer including age, fertility, and socioeconomic status. The pesticide use data were evaluated for the years 1970–1988 at the county level. The study reported a statistically significant 16–18% elevation in risk of breast cancer among Latinas in the highest exposure quartiles for methoxychlor. A more recent cross-sectional study from Egypt showed an increased level of methoxychlor in breast tumour tissue as compared to the normal surrounding tissue from 2013 and 2014 (OR 4.5) (Eldakroory *et al.*, 2017). Seventy female breast cancer patients ranging from 29 to 58 years of age had tissue samples taken. Organochlorine pesticides in samples were

measured, and oncogenic and apoptotic markers were analysed. Higher concentrations of methoxychlor were found in the tumour tissue samples compared to the normal surrounding tissue, and were statistically significant. Methoxychlor, had a weakly positive statistically significant correlation with G2m (a checkpoint prior to cell mitosis) and between annexin V (early apoptosis) and methoxychlor. Methoxychlor also showed a negative correlation with the markers PIM1, and Bcl-2. These epidemiological studies show an association between methoxychlor exposure and breast cancer incidence. However, a definitive conclusion on the relationship between methoxychlor exposure and breast cancer is not possible due to exposure to multiple pesticides and multiple risk factors.

143. A cross-sectional epidemiology study of adults from a rural village (Cidade dos Meninos) in Rio de Janeiro (Southeast Brazil) near a closed pesticide factory with abandoned stocks found differential effects on males and females associated with OCPs exposure (Freire *et al*., 2013). The study population included 303 men and 305 women (mean age of 39 years old) and was conducted between November 2003 and March 2004. Intravenous blood samples were collected for analysis of serum concentrations of each OCPs and levels of thyroid hormones (total triiodothyronine (T3) and free thyroxine (T4), thyroid-stimulating hormone (TSH), anti-thyroperoxidase (TPOAb) and anti-thyroglobulin (TgAb)). The LOD was 0.02 ng/mL for methoxychlor. Higher concentrations of methoxychlor exposure were significantly associated with increased total T3 levels in women. The detection of methoxychlor in the serum of men was associated with a statistically significantly increased odds of having TPOAb levels greater than 10 U/mL (OR = 2.19). TPOAb levels may be a useful indicator of thyroid injury or inflammation.

2.4.2.2 Neurotoxicity

144. Animal studies suggest that higher levels of exposure to methoxychlor can produce neurological effects, such as apprehension, nervousness, increased salivation, decreased locomotor activity, tremors, convulsions, and death (in rats $LOAEL_{oral}$ of 2,500 mg/kg/day (decreased locomotor activity) and 3,000 mg/kg/day (tremors); Cannon Laboratories, 1976 as cited in ATSDR, 2002). Methoxychlor undergoes demethylation to form phenolic derivatives, with dechlorination and dehydrochlorination reactions occurring to a lesser extent. (ATSDR 2002). In people with compromised liver function, neurological signs may occur at lower methoxychlor exposure levels. Methoxychlor has been demonstrated to be a neurotoxicant even in the absence of metabolic transformation. This suggests that it is the parent compound that is neurotoxic, and that neurotoxicity is of concern only when the metabolic capacity for O-demethylation is exceeded. This is supported by the observation that the neurological effects of methoxychlor are similar to those associated with exposure of humans and animals to DDT, a structurally similar chemical that is very slowly metabolized. The mechanism by which DDT, and therefore possibly methoxychlor, produces neurological effects has been proposed to involve the membrane-association of a lipophilic species, which alters ion transport across neural membranes (ATSDR, 2002).

145. In a later study by Golub *et al.* (2004), female rhesus monkeys (*n*=8/group) received daily oral doses of DES (0.5 mg/kg), and methoxychlor (MXC) (25 or 50 mg/kg) for 6 months before and after the anticipated age of menarche. Behavior was assessed during and for 9 months after dosing. Visual discrimination performance (simultaneous non match-to-sample with trial-unique stimuli) conducted during dosing demonstrated delayed improvement and poorer performance in the MXC50 group, with some similar effects in the DES group. Visual recognition memory, assessed with delays of ≤ 3 s, was not apparently affected. Spatial working memory, assessed after dosing, also showed acquisition deficits and possible working memory difficulties in the MXC50 group. Spontaneous motor activity, monitored at 6-month intervals, was not affected by treatment. Late peak latencies of the auditory brainstem response (ABR) were shorter in the DES group 6 months after treatment, suggesting long-term effects on brain. The study suggests that some aspects of brain function can be modified by exposure to exogenous estrogen during pubertal development. Although DES is a more potent estrogen, the high-dose MXC group was more affected behaviorally. According to the authors, differential effects of the two agents at the estrogen receptor subtypes (ERα and ERβ) may be relevant to the differential behavioral outcomes.

2.4.2.3 Endocrine disruption

146. According to US EPA (2004), methoxychlor has been discussed extensively in the scientific literature in connection with its effects on endocrine-mediated processes and disruption of the hypothalamic-pituitary-gonadal axis. Methoxychlor can be metabolized *in vivo* into two demethylated compounds (2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) and 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-dichloroethane (HPDE)) and two O-ring methylated compounds. These metabolites bind to the estrogen and androgen receptors. Therefore, methoxychlor may impair reproductive function through actions on estrogen and androgen receptors (Chen, 2014). For example, decreased serum progesterone levels may result from the estrogenic effects of methoxychlor on the ovaries that cause decreased follicular and corpus luteum development. The effects on the ovaries may be caused directly by methoxychlor metabolites or may result from effects on the pituitary and hypothalamus, which alter the release of regulatory hormones that affect the reproductive and accessory sex glands (ATSDR, 2002). Finally, studies with methoxychlor like the study of Gaido *et al.* (2000) demonstrate the complexity involved in determining the mode of action of methoxychlor metabolites that simultaneously act as an agonist or antagonist through one or more hormone receptors.

147. Primates, like humans, undergo a long and complex period of development during adolescence, which makes them important models for understanding exogenous estrogen effects during this period. Golub *et al.* (2003) examined

the effects of methoxychlor (25 and 50 mg/kg/day) and DES (0.5 mg/kg/day) during peripubertal period (6 months before and after the expected age at menarche) to female rhesus monkeys. Both treatments increased estrogen activity of serum (as determined with an *in vitro* estrogen receptor 1 (*ER1*) transcription assay). DES completely suppressed adolescent growth (weight and height) and menses in a reversible manner; smaller effects of methoxychlor (MXC) on the timing of growth and menarche were also detected. Both DES and MXC led to premature emergence of a secondary sex characteristic, reddening and swelling of skin, but retarded growth of the nipple. After an 8-month recovery period, uterine size was not affected by the treatments, but there was some indication of increased incidence of ovarian cysts/masses in the treated groups. Estrus cyclicity, as reflected in urinary hormone metabolites, demonstrated shorter follicular stages in the MXC-treated monkeys. In conclusion, the data indicate that DES had a striking effect on adolescent maturation and that MXC also altered development during this period. The pattern of effects across agents and doses may be based on specifics of estrogenic action, such as relative *ER1* and *ER2* binding and activation.

148. According to Gupta *et al.* (2007), methoxychlor (MXC) inhibits growth and induces atresia of antral follicles in rodents. MXC metabolites, mono-OH MXC (mono-OH) and bis-OH MXC (HPTE), are thought to be more toxic than the parent compound. Although studies have examined effects of MXC in rodents, few studies have evaluated the effects of MXC in primates. Gupta *et al.* (2007) investigated the effects of MXC, and its metabolites mono-OH, and HPTE on baboon antral follicles. Antral follicles were isolated from adult baboon ovaries and cultured with vehicle (DMSO), MXC (1-100 µg/ml), mono-OH (0.1-10 µg/ml), or HPTE (0.1-10 µg/ml) for 96 hr. Growth was monitored at 24 hr intervals. After culture, follicles were processed for histological evaluation of atresia. MXC, mono-OH, and HPTE significantly inhibited follicular growth and increased atresia compared to vehicle. Moreover, the adverse effects of MXC and its metabolites on growth and atresia in baboon antral follicles were observed at lower (100-fold) doses than those causing similar effects in rodents. These data suggest that MXC and its metabolites inhibit growth and induce atresia of baboon antral follicles, and that primate follicles are more sensitive to MXC than rodent follicles.

149. White *et al*. (2005) (as cited in ATSDR, 2012 addendum) demonstrate that methoxychlor (or its metabolites) have the capacity to impair the developing immune system *in vivo.* This study evaluated the immunotoxicity of methoxychlor in F0 (dams) and F1 generations of Sprague-Dawley rats exposed to an isoflavone-free diet containing methoxychlor at concentrations of 10, 100, and 1000 ppm. In dams, exposure to methoxychlor from gestation day 7 to postpartum day 51 (65 days total exposure) produced a significant increase in natural killer cell activity (1000 ppm) and in the percentages of T cells (1000 ppm), helper T cells (1000 ppm), and macrophages (100 and 1000 ppm). In contrast, a decrease in the numbers of splenocytes and B cells was observed at the 100 and 1000 ppm concentrations. In F1 males, exposure to methoxychlor gestationally, lactationally, and through feed from postnatal day 22–64 (78 days total exposure) produced an increase in the spleen IgM antibody-forming cell response to sheep red blood cells (100 and 1000 ppm) and the activity of NK cells (1000 ppm). However, there was a decrease in the terminal body weight (1000 ppm), spleen weight (1000 ppm), thymus weight (100 and 1000 ppm), and the numbers of splenocytes (1000 ppm), B cells (100 and 1000 ppm), cytotoxic T cells (1000 ppm), and NK cells (100 and 1000 ppm). In F1 females, exposure to methoxychlor produced a decrease in the terminal body weight (1000 ppm) and the percentages of cytotoxic T cells (10, 100, and 1000 ppm). These results demonstrate that developmental and adult dietary exposure to methoxychlor modulates immune responses in Sprague-Dawley rats. Immunological changes were more pronounced in the F1 generation male rats that were exposed during gestation and postpartum than in the F0 and F1 generation females. Increases in antibody-forming cell response and NK cell activity and altered spleen cell subpopulation numbers were observed in the F1 generation male rats, without similar changes to the F1 generation females. According to the authors, the endocrine disrupting properties (estrogenic effects) of methoxychlor might explain why exposure to methoxychlor had significant effects on the immune parameters in F1 male rats, while it had minimal effects in F0 and F1 female rats. Similarly, the findings of the *in vitro* study of Leung-Gurung *et al.* (2018) suggest that the metabolite of methoxychlor, HPTE, may play a pivotal role in metoxychlor exposure-induced immune dysfunction. This study is further discussed in the Appendix.

150. Progesterone and estradiol produced by the human placenta are critical for maintenance of pregnancy and fetal development. In the human placenta, 3 beta-hydroxysteroid dehydrogenase 1 (HSD3B1) is responsible for the formation of progesterone from pregnenolone and aromatase (CYP19A1) for the production of estradiol from androgen. According to Liu *et al.* (2016), methoxychlor and its metabolite hydroxychlor (HPTE) may disrupt the activities of these 2 enzymes. Liu *et al.* (2016) investigated the effects of methoxychlor and HPTE on steroid production in human placental JEG-3 cells and on HSD3B1 and CYP19A1 activities. Methoxychlor and HPTE inhibited progesterone and estradiol production in JEG-3 cells. Methoxychlor and HPTE were potent HSD3B1 inhibitors with the half maximal inhibitory concentration (IC_{50}) values of 2.339 ± 0.096 µmol/L (808.476 ± 33.182 µg/L) and 1.918 ± 0.078 µmol/L (609.138 ± 31.759 µg/L), respectively. Methoxychlor had no inhibition on CYP19A1 at 100 µmol/L (34.565 mg/L), while HPTE was a weak inhibitor with IC50 of 97. 16 ± 0.10 µmol/L (30857 ± 31.759 µg/L). When pregnenolone was used to determine the inhibitory mode, methoxychlor and HPTE were found to be competitive inhibitors of HSD3B1. When cofactor NAD(+) was used, methoxychlor and HPTE were the noncompetitive inhibitors of HSD3B1. When testosterone was used, HPTE was a

mixed inhibitor of CYP19A1. The authors concluded that methoxychlor and its metabolite HPTE are potent inhibitors of human HSD3B1, and HPTE is a weak CYP19A1 inhibitor.

2.4.2.4 Interaction with other chemicals

151. Nishino *et al.* (2014) demonstrated combined effects of three well-known environmental immunotoxic chemicals – methoxychlor (MXC), an organochlorine compound; parathion (PARA), an organophosphate compound; and piperonyl butoxide (PBO), an agricultural insecticide synergist – by short-term oral gavage exposure. Seven-week-old Balb/cAnN mice received daily oral gavage exposure to either one or two of the environmental immunotoxic chemicals for 5 consecutive days. On Day 2, all mice in each group were immunized with sheep red blood cells (SRBC), and their SRBC-specific IgM responses were analyzed by using an enzyme-linked immunosorbent assay and plaque-forming cell assay. T- and B-cell counts in the mouse spleens were also assessed via surface antigen expression. Mice that received MXC + PARA and PBO + MXC treatment showed marked decreases in SRBC-specific IgM production (both MXC + PARA and MXC + PBO resulted in additivity in the serum only) and T- and B-cell counts (In the case of T-cells, and total B-cell counts, only MXC + PARA resulted in additivity and in the case of PNA^+ B-cells, only MXC + PBO resulted in additivity) compared with those in mice that received vehicle control or the corresponding individual test substance. This suggests that simultaneous exposure to multiple environmental chemicals increases the immunotoxic effects of the chemicals compared to individual exposures.

152. Embryonic exposure to estrogens and estrogenic pollutants is known to demasculinize sexual behaviour in adult male Japanese quail. Halldin *et al.* (2005) administered methoxychlor to quail eggs before sexual differentiation of the brain at a dose of 150 µg/g egg and then studied sexual behaviour and other reproductive variables in adult males. In a second experiment, the authors administered the same dose of methoxychlor together with 10 µg/g egg of the commercial polychlorinated biphenyl (PCB) mixture Clophen A50 (CA50) and also CA50 alone. Neither methoxychlor nor CA50 had any significant effects by themselves, but when they were administered together significant changes in male sexual behaviour were observed, specifically a significant reduction ($p=0.0010$) in the number of successful mount attempts and cloacal contact movement. According to the authors, it seems likely that induction of biotransformation enzymes in the embryos by CA50 resulted in increased conversion of methoxychlor to the more estrogenic metabolite 2,2-*bis*(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE).

153. In mice receiving 25 mg/kg/day methoxychlor by gavage in an olive oil suspension along with 12 mg/kg/day bromfenvinphos for 6 weeks, inflammatory infiltrations of the liver were larger and denser than observed in mice receiving bromfenvinphos alone (Zaleska-Freljan *et al.*, 1983 as cited in ATSDR, 2002). Small changes were observed in the kidneys at similar frequencies and severities in both treatment groups. In this study, a methoxychlor-only group was not used. The liver inflammatory infiltrations observed in this study have not been observed in other studies limited to
methoxychlor-only exposures and thus could be interpreted as resulting from the combined exposures of methoxychlor and bromfenvinphos (ATSDR, 2002).

154. The joint toxic actions of binary mixtures of methoxychlor (MXC) and other pesticides (including organophosphates and other chlorinated hydrocarbons) on acute lethality were examined in mice (Keplinger and Deichmann, 1967 as cited in ATSDR (2002)). After determining oral LD_{50} values for the individual compounds, binary mixtures (with components at equitoxic doses based on LD_{50} values) suspended in corn oil were administered to fasted mice via gavage at the same dose ranges as the individual compounds. Based on the assumption of joint additive action, an expected LD_{50} value was calculated for each mixture and compared with the observed LD_{50}. The authors considered biological variation and other factors and chose ratios of ≤ 0.57 to be indicative of a significantly reduced degree of toxicity, or antagonism, and ratios of ≥ 1.75 as indicative of synergism. The ratio of expected:observed LD_{50} values for the MXC/DDT mixture (0.66) indicated a less than additive action (i.e., mutual protection). Ratios for the MXC/aldrin (0.81), MXC/diazinon (0.82), MXC/malathion (0.84), MXC/toxaphene (0.92), and MXC/aramite (1.25) mixtures were close to 1.00, indicating joint additive action. Ratios for the MXC/parathion (1.51), MXC/delnav (1.96), MXC/dieldrin (2.06), and MXC/chlordane (2.26) mixtures were suggestive of greater than additive joint action (i.e., potentiation or synergism).

155. When methoxychlor was administered orally to rats previously treated with carbon tetrachloride, DDT-like neurological symptoms were observed (Lehman, 1952 as cited in ATSDR, 2002). In addition, methoxychlor was found to accumulate in the fat and liver in amounts approximately 15–19 times the levels observed in control animals. Carbon tetrachloride is known to inactivate certain hepatic enzymes (CYPs or cytochrome P450s) which metabolize xenobiotics, thereby increasing their retention. These data suggest that carbon tetrachloride and other chemicals that inhibit the metabolism of methoxychlor may increase the risk of neurotoxicity (ATSDR, 2002).

2.4.2.5 Summary of adverse effects on human health

156. Based on the available experimental studies, it is not possible to draw definitive conclusion as to whether methoxychlor is carcinogenic to animals or humans. Epidemiological studies, cited above, have suggested an association but have not definitively shown causation with respect to the carcinogenicity potential of methoxychlor due to study limitations. Based on animal data genotoxic potential of methoxychlor appears to be negligible. Animal

and *in vitro* data strongly suggest that methoxychlor may adversely affect the development, histopathology, and function of the human reproductive system (likely through an estrogenic mode of action). Reproductive effects are indicative of interference with the normal actions of estrogen or androgen. Methoxychlor has been demonstrated to induce behavioral changes in primates. In certain defined cases, the simultaneous exposure of methoxychlor to other environmental chemicals has resulted in additive effects.

3. Synthesis of information

157. Methoxychlor is an organochlorine pesticide that has been used as a replacement for DDT. It has been restricted/banned in several countries for more than 15 years. In response to the call for information (Annex E information submission (2019)), no Parties have indicated current use of methoxychlor. However, a literature search made for the purpose of drafting the risk profile suggests that recent use of methoxychlor may have occurred in certain countries. In 1975, three U.S. companies produced 2500 tonnes of methoxychlor. The production decreased to 193 tonnes in 1991. After 1992, production of methoxychlor in the U.S. was significantly reduced until its ban in 2000. No information on the current production or use of methoxychlor at a global scale is publicly available. Methoxychlor does not occur naturally in the environment. It is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Smaller amounts of methoxychlor may be released to the environment during its production, formulation, storage, shipment and disposal. Based on a maximum historical estimate of 8,000 tonnes/year of methoxychlor produced worldwide (circa 1975), peak historical atmospheric releases during production were estimated to be up to 4 tonnes/year. In the U.S., a total of 1.04 tonnes of methoxychlor was released in 2018 from on- and off-site disposal (or other releases) (US EPA, 2020a).

158. Methoxychlor is highly hydrophobic. Based on its physicochemical properties, it will most likely distribute to the sediment and biota. Methoxychlor is expected to be tightly bound to soil particles. However, soil particles that contain it can be blown by the wind or be carried by rainwater or melted snow into rivers or lakes. In addition, groundwater monitoring data suggest that some leaching of methoxychlor may take place in soil. Based upon its relatively low vapour pressure value, methoxychlor has a low potential for volatilisation to the atmosphere. However, concentrations found in remote regions suggest its atmospheric transport.

159. Modelling data (BIOWIN 2, 3 and 6) indicate that methoxychlor is not expected to biodegrade fast, and hence is potentially persistent. Methoxychlor hydrolysis is considered to be negligible. Although photolysis rates of methoxychlor seem moderate, photolysis is not expected to contribute to the degradation significantly as photolysis in water only takes place in the top layers of the water column. Methoxychlor may undergo photolysis on soil surfaces, which is based on studies reporting the photolysis of dry methoxychlor films exposed to sunlight and on the photodegradation of a structural analogue (ethoxychlor) in soil. However, methoxychlor photodegradation in soil at depths below a few millimetres is not expected to happen. Based on a weight-of-evidence approach, the results from the laboratory study and monitoring data indicate that methoxychlor is persistent in aerobic sediments and may be persistent in some anaerobic sediments. Measured data have shown that methoxychlor continued to be detected in surface waterbodies in Europe and Canada, and in French groundwaters, years after it was phased out, thus providing some evidence that the substance is persistent in water. Monitoring data from an Arctic lake and surface seawater in a region covering the North Pacific to the Arctic Ocean, further indicate that the substance may be persistent in the surface water and marine water compartments. Based on a weight-of-evidence approach, the results from the laboratory studies and monitoring data indicate that methoxychlor may be persistent in some aerobic soils. However, the presence of methoxychlor in the surface water, seawater and soil mentioned above can also be a result of long-range transport.

160. Methoxychlor is a lipophilic substance with an experimental log K_{ow} value of 5.08. The BAF value (9001 L/kg) predicted by the Arnot-Gobas method (upper trophic) suggest bioaccumulation potential of methoxychlor in aquatic organisms (BAF > 5,000). BCF values for methoxychlor vary largely between different aquatic species as a result of species differences in the capacity to metabolise and excrete methoxychlor (BCFs in the range of 667–8,300). Laboratory studies indicate that methoxychlor has a bioaccumulation potential in some fish species with BCF values > 5,000. In addition, supporting information such as a BCF value of 12,000 in a bivalve *Mytilus edulis* and an average BCF of 6,945 (range of 5,000 to 8,570) in the snails *Physa integra* indicate that methoxychlor has bioaccumulation potential in aquatic invertebrates. Combination of the bioaccumulation potential of methoxychlor with a high toxicity and high ecotoxicity gives reason for concern. Toxicokinetic and metabolism studies would suggest that methoxychlor does not accumulate in mammals.

161. Methoxychlor is expected to exist in both the particulate phase (bound to particulate matter) and, to a small degree, the vapour phase in the atmosphere. Despite modelling predictions indicating a low LRTP, transport of methoxychlor to remote areas (Arctic and Antarctica) is taking place based on monitoring data. The presence of methoxychlor in remote areas can be explained by atmospheric transport in the gas phase or on particles during dry periods and during periods of lower photolytic activity. Furthermore, considering the persistence of the substance in water (measurements in surface waterbodies from Europe and Canada after its ban), and that methoxychlor, endosulfan and pentachloroanisole were found at collective concentrations in the range of 0.017–0.023 ng/L in an Arctic lake, as well as the measured level of methoxychlor in the Arctic Ocean and sea (concentrations in the range

<MDL–0.38 ng/L (mean 0.15±0.11 ng/L), long-range transport potential through water and ocean currents is also possible. In the absence of known local or regional pesticide sources, detection in environmental and biota samples from the Arctic and Antarctica are resulting from long-range environmental transport of methoxychlor.

162. Methoxychlor has been frequently detected in the environment and in biota globally. It has been detected in numerous environmental matrices worldwide including in the Arctic (in air, snow, ice core, lake waters and seawater, in biota samples (terrestrial, avian and marine)) and in Antarctica (in marine biota samples and in milk of elephant seals). In 2016 and 2017, among three monitored insecticides, methoxychlor was one of the most abundant insecticides in the atmosphere. In addition, from 1993 to 1995, it was found in the Arctic air at higher concentration than the POP, endrin. Environmental trend data are not available and monitoring data are insufficient to conclude on a trend. However, from 1999 to 2014, it was observed that the concentrations of methoxychlor in Southern elephant seals in Antarctica increased. No data are available on trends of methoxychlor in humans.

163. Humans are exposed to methoxychlor mainly through intake of contaminated food and drinking water but also through the indoor environment via exposure to dust and aerosols in air and through oral uptake of dust and soil. Small children may play close to the ground and are therefore more likely than adults to come in contact with dirt and dust. Children also may intentionally or unintentionally ingest dust or soil that contains low levels of methoxychlor. Methoxychlor has been detected in human serum, adipose tissues, umbilical cord blood and human breast milk. Children can be exposed to methoxychlor *in utero* via the placenta and after birth via lactation.

164. Methoxychlor is very toxic to aquatic invertebrates and fish. Freshwater arthropods are the most acutely sensitive species with a HC_5 of 0.37 µg/L (or 370 ng/L). Methoxychlor is suspected to have endocrine disruptive effects in fish, amphibian, and sea urchin fertility, growth, and development. Methoxychlor is practically acutely or subacutely nontoxic to birds, but reproductive toxicity has not been tested. Animal studies indicate that high doses of methoxychlor are capable of causing neurological injury (tremors, convulsions), but most studies indicate that the reproductive system is the most sensitive target for methoxychlor. The resultant types of reproductive effects are indicative of interference with the normal actions of estrogen or androgen. In addition, developmental and adult dietary exposure to methoxychlor in rats modulates immune responses. Furthermore, observations in rats indicate that methoxychlor has the potential to promote the epigenetic transgenerational inheritance of disease and associated sperm epimutations.

165. In humans, methoxychlor is not recognised as a carcinogen, although epidemiological studies have suggested an association, but have not shown causation due to study limitations. Based on animal data, genotoxic potential of methoxychlor appears to be negligible. However, animal and *in vitro* data strongly suggest that it may adversely affect the development, histopathology, and function of the human reproductive system (likely though an estrogenic mode of action). Reproductive effects are indicative of interference with the normal actions of estrogen or androgen. At high doses methoxychlor has been demonstrated to be a neurotoxicant. At lower doses alteration of cognitive functions in sensitive life stages in primates were reported. In certain defined cases, the simultaneous exposure of methoxychlor and other environmental chemicals has resulted in additive effects. A TDI value of 0.005 mg/kg/day[19] for methoxychlor was derived by WHO (2004) based on the NOEL of 5.01 mg/kg/day for maternal toxicity in rabbits. Allocation of 10% of the TDI to drinking-water results in a guideline value of 20 µg/L.

166. The level of methoxychlor found in the drinking water from Slovakia exceeded the EU general quality standard for individual pesticides. Comparison of measured levels of methoxychlor in the environment or in human food with (eco)toxicity data indicate that some of the concentrations found in human food and surface water were in excess compared to RfD or PNEC values derived by the authors. This suggests a potential risk for human populations and for aquatic organisms. However, it is worth noting that for POP substances an acceptable threshold in the environment cannot be established using the methods currently available with sufficient reliability for an acceptable risk to be determined in a quantitative way. Indeed, experience with POP substances has shown that they can give rise to specific concerns that may arise due to their potential to accumulate in parts of the environment and that the effects of such accumulation are unpredictable in the long-term. Cessation of emissions will not necessarily result in a reduction in substance concentration and therefore such accumulation is in practice difficult to reverse.

167. Furthermore, POP substances may have the potential to contaminate remote areas that should be protected from further contamination by hazardous substances resulting from human activity because the intrinsic value of pristine environments should be protected. These specific concerns occur particularly with substances that can be shown to persist for long periods and to bioaccumulate in biota and which can give rise to toxic effects after a longer time and over a greater spatial scale than substances without these properties. These effects may be difficult to detect at an early stage because of long-term exposures at normally low concentration levels, and long life-cycles of species at the top of the food chain. Thus, concentrations of methoxychlor in biota from the Arctic and Antarctica and in humans indicate a potential for adverse effects in wildlife and in humans. Methoxychlor was also found: in the eggs of a critically endangered sea turtle, in the fat tissues of crocodiles living in a biodiversity hotspot in South Africa and in

[19] It is worth noting that the ATSDR MRL (ATSDR, 2002) and the US EPA RfD (IRIS, 2003) were also set at 0.005 mg/kg/day.

the air of two national parks in Southeast Brazilian mountains where hundreds of endangered species and many endemic species live thus suggesting a potential concern for these species.

168. Based on its inherent properties, methoxychlor is likely to lead to significant adverse environmental effects and may lead to significant adverse human health effects, such that global action is warranted.

4. Concluding statement

169. Methoxychlor does not occur naturally in the environment. It is released to the environment mainly as a result of its application to crops and livestock as a pesticide. Smaller amounts of methoxychlor may be released to the environment during its production, formulation, storage, shipment and disposal. Methoxychlor is persistent, bioaccumulative, toxic to aquatic organisms and terrestrial animals (including humans) and undergoes long-range environmental transport, making emissions of this substance a transboundary pollution problem including in remote areas. Globally, the occurrence and distribution of methoxychlor is shown for humans, wildlife and the environment. Detections include measurements in the Arctic and Antarctic.

170. Methoxychlor has been frequently detected in the environment and in biota globally. It has been detected in numerous environmental matrixes worldwide including in the Arctic (in air, snow, ice cores, lake waters and seawater, in biota samples (terrestrial, avian and marine)) and in Antarctica (in marine biota samples and in milk of elephant seals). Methoxychlor has been detected in human: serum, adipose tissue, umbilical cord blood and breast milk. It has also been found in human food including in drinking water and groundwater.

171. The concern for adverse effects includes toxic effects on reproduction, including potential endocrine disruption, modulation of immune responses and a potential for promoting epigenetic transgenerational inheritance of diseases and associated sperm epimutations and alteration of cognitive functions. Methoxychlor is also toxic to aquatic organisms. Due to its POP properties, concentrations of methoxychlor in biota from the Arctic and Antarctica and in humans indicate a potential for adverse effects in wildlife and in humans. Methoxychlor was also found: in the eggs of a critically endangered sea turtle, in the fat tissues of crocodiles living in a biodiversity hotspot in South Africa and in the air of two national parks in Southeast Brazilian mountains where hundreds of endangered species and many endemic species live thus suggesting a potential concern for these species.

172. Methoxychlor has been restricted/banned in several countries for more than 15 years. However, the outcome of a literature search suggests that recent use of methoxychlor may have occurred in certain countries. Since methoxychlor demonstrates persistence and long-range transport, measures taken nationally or regionally are not sufficient to safeguard a high level of protection of the environment and human health, and therefore wider international action is necessary.

173. Based on the persistence, bioaccumulation, toxicity to aquatic organisms and in terrestrial animals (including humans) and the widespread occurrence in environmental compartments including remote regions, it is concluded that the use of methoxychlor is likely to lead to significant adverse human health and environmental effects such that global action is warranted.

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Appendix

Note: This Appendix contains additional information to chapter 2 of the risk profile for methoxychlor.

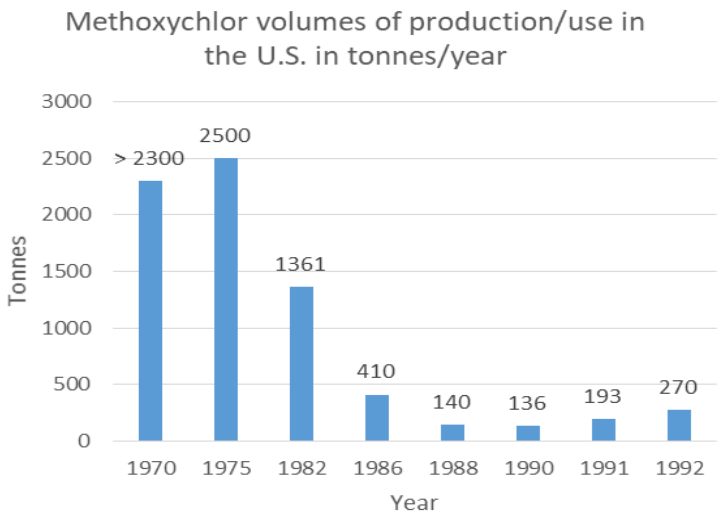
2. Summary information relevant to the risk profile

2.1 Sources

2.1.1 Production, trade, stockpiles

2.1.2 Uses

a)



b)

Late 1970s to early 1980s	1986	1990-1991	1995	References
> 2300 t/y (U.S. production)	230–410 tonnes (use in the U.S.)	136–181 t/y (use in the U.S.)	0.54 tonnes (use in California)	Götz et al., 2008
2495 tonnes (in 1975) (U.S. production)				
8.03 tonnes (in 1978) (imported in the U.S)	226.8-408.2 tonnes (use in the U.S)	193 tonnes (in 1991) (production)		ATSDR, 2002
1361 tonnes (in 1982) (U.S. production)				
		140-270 t/y (between 1988-1992) (U.S. production)		Minister of Indian Affairs and Northern Development Ottawa, 2003

Figure 1a) and b): Methoxychlor production/use/import volumes in tonnes per year in the U.S. (Götz *et al.*, 2008; ATSDR, 2002; Minister of Indian Affairs and Northern Development Ottawa, 2003)

2.1.3 Releases to the environment

2.2 Environmental fate

2.2.1 Environmental distribution

Adsorption/desorption

1. Methoxychlor is sparingly soluble in water with a water solubility which is in the range of 0.04–0.302 mg/L (see references in Table 3). Based on its log K_{ow} value of 5.08 (experimental Karickhoff *et al.*,1979) and 5.67 (predicted with KOWWIN v1.68 estimate; US EPA, 2012), the substance is expected to be hydrophobic with low water solubility and a high adsorption coefficient. Measured average K_{oc} values in pond and river sediments are as follows: 23,000 in sand, 82,000 in coarse silt, 88,000 in medium silt, 93,000 in fine silt and 83,000 in clay (Karickhoff *et al.*,1979). These K_{oc} values indicate that methoxychlor is expected to adsorb strongly to suspended solids and sediment. Muir and Yarechewski (1984) observed that methoxychlor partitioned rapidly (< 3 days) into sediment following addition to sediment-water systems. In aquatic environments, it is suggested, based on the physicochemical properties discussed here, that methoxychlor will most likely concentrate in sediment and biota.

2. Weber *et al.* (2004) estimated a mean sorption coefficient (K_d) of 2009 in soil for methoxychlor (number of values used for the calculation: 11) which indicates that methoxychlor is tightly bound to soil particles. Modelling data are in agreement with experimental data. EPI Suite KOCWIN v2.00 model (KOCWIN, MCI method; US EPA, 2012) predicts a K_{oc} value of 26,890 L/kg for methoxychlor (equivalent to a log K_{oc} value of 4.43). An experimental log K_{oc} value of 4.9 (Schüürmann *et al.*, 2006) is also reported in EPI Suite (US EPA, 2012). Both of these log K_{oc} values also indicate that methoxychlor is expected to be immobile in soil. However, soil particles that contain methoxychlor can be blown by the wind or be carried by rainwater or melted snow into rivers or lakes (ATSDR, 2002).

Volatilisation

3. Methoxychlor has an experimental Henry's Law constant of 2.03 x 10^{-7} atm.m^3/mol (or 2.06 x 10^{-2} Pa·m^3/mol; wetted wall column-GC) at 25°C (US EPA, 2012). The estimated Henry's Law constant for methoxychlor is 9.75 x 10^{-8} atm.m^3/mol (or 9.88 x 10^{-3} Pa·m^3/mol) at 25°C using EPI Suite HENRYWIN v. 3.20 model (bond method; US EPA, 2012). Both experimental and predicted Henry's Law constant values indicate that methoxychlor can be expected to be non-volatile from water. Furthermore, Bomberger *et al.* (1983) modelled the transport and fate pathways for methoxychlor at the air-terrestrial interface, and the model simulations suggest that the compound is strongly adsorbed to soil and does not leach, and that volatilisation is slow. Based upon its relatively low vapour pressure value (5.56 x10^{-3} Pa at 25°C (EPI Suite, MPBPVP v1.43 estimate, modified grain method; US EPA, 2012), methoxychlor has a low potential for volatilisation to the atmosphere. However, concentrations of methoxychlor found in remote regions indicate that atmospheric transport is occurring.

Distribution modelling

4. The Mackay Level III Fugacity Model in EPI Suite (US EPA, 2012) was used in order to model the distribution of methoxychlor. The physico-chemical properties used in the model were the experimental values (if not stated otherwise) reported in Table 3: melting point 87°C; boiling point 346 °C, vapour pressure 5.56×10^{-3} Pa (modelled value); Henry Law Constant 2.06×10^{-2} Pa; water solubility 0.1 mg/l; log K_{ow} 5.08; K_{oc} value 79433 (log 4.9). The degradation half-lives used in the model environment are: air 4.8 hours (or 0.2 days derived by AOPWIN); water 4992 hours (or 208 days (Walker *et al.*, 1988); soil 5040 hours (or 210 days (Guth *et al.*, 1976)); sediment 4944 hours (or 206 days (Muir and Yarechewski, 1984)). If methoxychlor is assumed to be released at equal rates to air, water and soil, the model predicts the following distribution: air 0.058 %, water 6.12 %, soil 75.8% and sediment 18%, indicating that the substance will be mainly distributed to the soil and the sediment. If it is released only to soil, the calculated distribution is very different: air 1.29 ×10^{-5} %, water 0.0143 %, soil 99.9% and sediment 0.0422%, predicting that the substance will stay in the soil compartment. It should be noted that there is uncertainty in the half-lives in water, in soil and in air.

2.2.2 Persistence

2.2.2.1 Screening information based on modelling data

5. The aerobic biodegradation potential of the substance can be assessed using BIOWIN v4.10 (U.S. EPA, 2012). The predictions from the non-linear model (BIOWIN 2), ultimate biodegradation time (BIOWIN 3) and the MITI non-linear model (BIOWIN 6) can be used as a screening assessment of persistence in accordance with the REACH Guidance Chapter R.11 (ECHA, 2017). The following screening information indicate when a substance may be 'persistent or very persistent':

(a) BIOWIN 2: 'Does not biodegrade fast' (probability < 0.5) and BIOWIN 3: ≥ months (< 2.25 (to 2.75)) or

(b) BIOWIN 3: ≥ months (< 2.25 (to 2.75)) and BIOWIN 6: 'does not biodegrade fast' (probability < 0.5).

6. The predictions for the structure of methoxychlor are BIOWIN 2: 0.0162, BIOWIN 3: 1.5126 and BIOWIN 6: 0.0063. These values are all well below the screening values, indicating that methoxychlor is not expected to biodegrade rapidly and hence is potentially 'persistent or very persistent'.

2.2.2.2 Abiotic degradation

2.2.2.3 Biotic degradation

7. Walker *et al* (1988) investigated the first-order biotic and abiotic degradation rate constants of methoxychlor in estuarine water and sediment/water slurry systems. Test systems used a methoxychlor nominal concentration of approximately 200 µg/L in sterile and non-sterile samples of water and water-sediment systems taken directly from the field (Florida, USA). Either acetone or hexane was used as a carrier solvent to prepare the test solutions (concentration not specified). Sterile samples were prepared by addition of 20ml/L 37% (by weight) formaldehyde solution. Salinity ranged from 15 to 30 g/L. Sediment samples were collected from the top 2 cm of the sediment bed (reflecting aerobic conditions). Flasks containing water-only and water-sediment slurries were placed in a shaker incubator and incubated in the dark at 25°C. The pH was measured at least every other day and was maintained to within ± 0.2 units of the initial pH of the water sample collected. Duplicate samples were removed periodically from each flask and analysed for residual test chemical (non-labelled chemical). Control sediment and water systems (no test chemical or formaldehyde added) were used. Analytical recoveries were > 85%. The first-order rate constant (k1) for methoxychlor was determined. Identification of degradation products was not studied, only the parent substance was followed in the test. Carbon dioxide formation was not measured in the experiment. The methoxychlor half-lives derived from first-order rate constants in water-only systems were 208 and 8,830 days for non-sterile and sterile conditions, respectively. The derived methoxychlor half-lives derived from first-order rate constants in water-sediment systems were 12.2 and 45.2 days at 25°C for non-sterile and sterile conditions, respectively (under aerobic conditions). The results of this study indicate significantly ($p \leq 0.01$) more degradation in the presence of non-sterile sediment than in the presence of sterile sediment (indicating biotic degradation), and that methoxychlor biodegraded significantly faster in flasks containing non-sterile water-sediment systems than in non-sterile water alone. The presence of non-extracted methoxychlor from sediment was not taken into account in the calculation of the half-lives and the calculated half-lives may therefore not represent the most conservative case for methoxychlor.

8. The study was performed prior to publication of the Organisation for Economic Co-operation and Development (OECD) 309 test guideline and therefore cannot be directly assessed against their recommended test design and validity criteria. The nominal water-only concentration exceeds the recommended test concentration of the TG 309 (100 µg/L), and only one concentration was tested. It is not clear whether the test concentration was above the limit of solubility of methoxychlor in the test water. A reference substance and a solvent control were not used. As a consequence, it is not possible to assess the microbial activity and potential adverse effects of the solvent on the microorganisms in the test, although it is noted that solvent was removed by flushing the glass bottles used to prepare the test systems with air or nitrogen prior to adding site water. In addition, the degradation curve and raw data for methoxychlor are not reported and no information is available on the mass balance. It is not possible to say if these values reflect dissipation or degradation half-lives. Due to these significant variations from the current guideline simulation studies (and consequent limitations), the study results cannot be considered as reliable. However, the half-life in water of 208 days has been used as input data for the distribution modelling (Mackay Level III Fugacity Model) and for the OECD *P*ov and LRTP Screening Tool as this is the best information on half-life available and it is line with monitoring data available on surface waters.

2.2.2.4 Monitoring data

2.2.2.5 Summary on persistence

2.2.3 Bioaccumulation

2.2.3.1 Screening information based on modelling data

9. The bioaccumulation model (BCFBAF v3.01; US EPA, 2012) estimated a bioconcentration factor (BCF) value of 1044 L/kg wet-wt (based on the regression-based method and using as input data an experimental log K_{ow} value of 5.08), a BCF value of 4134 L/kg and a bioaccumulation factor (BAF) of 9001 L/kg (based on the Arnot-Gobas method (upper trophic) and using as input data an experimental log K_{ow} value of 5.08). The prediction can be considered reliable as the substance is within the applicability domain of the model (both structural and parametric). It is further noted that the somewhat structurally similar compound DDT (CAS 50-29-3) is in the training set of the model and methoxychlor is in the validation set of the model (BCF 315 L/kg wet-wt). The predicted BAF value suggests a bioaccumulation potential of methoxychlor in aquatic organisms (BAF > 5000).

2.2.3.2 Bioconcentration and bioaccumulation studies in aquatic organisms

2.2.3.3 Toxicokinetic and metabolism studies

10. A summary of methoxychlor metabolic pathways is presented in Figure 3-2 of ATSDR, 2002.

2.2.3.4 Conclusion on bioaccumulation

2.2.4 Potential for long-range environmental transport

2.2.4.1 Screening of physicochemical properties

2.2.4.2 Long-range transport model predictions

11. Overall persistence (*P*ov) and the potential for long-range transport (LRTP) can be estimated for organic chemicals using the OECD *P*ov and LRTP Screening Tool (Wegmann *et al*., 2009). Table 4 shows a summary of input data for methoxychlor to the OECD *P*ov and LRTP Screening Tool.

Table 4: Input properties used in estimating overall persistence and long-range transport potential of methoxychlor with the OECD *P*ov and LRTP Screening Tool

Input Property	Value	References	Comments
Log K_{aw}	-5.081 *(EPI Suite, KOAWIN v1.10, calculated from experimental Henry's Law Constant)*	US EPA, 2012	
Log K_{ow}	5.08 *(experimental, in KOWWIN training set)*	Karickhoff *et al*.,1979	
Half-life in air (in the gas-phase)	4.8 hours (or 0.2 days) *(estimated based on a 12-h photoperiod, AOPWIN v1.92)*	US EPA, 2012	
Half-life in water	4992 hours (or 208 days) *(experimental)*	Walker *et al.*, 1988	Uncertainty on this value as this study is considered to be not reliable.
Half-life in soil	5040 hours (or 210 days)	Guth *et al.*, 1976	Uncertainty on this value as experimental details are not available in Guth *et al.*, 1976.

12. Whilst no absolute criteria for classifying chemicals as compounds with high or low overall persistence (*P*ov) and LRTP have been established, the OECD expert group proposed making comparative assessments based on a set of substances selected as reference compounds. *P*ov and LRTP results for the reference substances can then be used to provide comparative context for other substances. Klasmeier *et al.* (2006) have described this approach in detail. Using the model results for the reference substances Klasmeier *et al.* (2006) defined four areas in the plot of LRTP vs. *P*ov. The *P*ov value of the POP-like reference substance with the lowest *P*ov result defines the boundary between high and low *P*ov; the LRTP value of the POP-like reference substance with the lowest LRTP result defines the boundary between high and low LRTP. This approach has been applied using the Tool to derive *P*ov and LRTP boundaries that can be used as reference points in screening chemicals. The *P*ov boundary is 195 days (*P*ov of α-HCH) and the LRTP boundaries are 5097 km (CTD of PCB 28) and 2.248 % (TE of PCB-28). Using the OECD *P*ov and LRTP Screening Tool (Wegmann *et al*., 2009) with input data specified in Table 4, the results obtained for methoxychlor suggest that it has a low potential for long-range transport: CTD of 498 km, *P*ov of 303 days and transfer efficiency from air to surface media of 0.02 %. However, the *P*ov of methoxychlor is higher (303 days) than the *P*ov of α-HCH (195 days). The results from this modelling are associated with uncertainty, because the input parameters "half-lives in water and soil" are uncertain.

2.2.4.3 Confirmation based on measurements in remote areas

2.2.4.4 Summary of long-range environmental transport

2.3 Exposure

2.3.1 Environmental monitoring data

2.3.1.1 Monitoring in remote areas (far from point sources)

2.3.1.2 Monitoring in rural and urban areas

13. Tremblay *et al.* (2017) studied concentrations of OCPs in the blood (plasma) of female hawksbill turtles (*Eretmochelys imbricata*) collected at Punta Xen (Campeche) in southern Gulf of Mexico. Samples were collected in August 2010. Methoxychlor was found in one third (n=10) of the sea turtles. Methoxychlor was detected in the blood (plasma + washed erythrocytes) of 10 out of 28 hawksbill sea turtles samples (36%) with concentrations in the range of 10–122 ng/g (mean: 23 ng/g; median: 11 ng/g; SD:29). Methoxychlor was the only OCP correlated with the size of the sea turtles. According to the authors, as methoxychlor can have endocrine disrupting properties, its relatively high presence in females nesting in Punta Xen is of concern.

14. García-Besné *et al.* (2015) studied concentrations of OCPs in the blood (plasma) and fertile eggs of two sea turtle species (*Eretmochelys imbricata* and *Chelonia mydas*) collected from Campeche in the Yucatan Peninsula in southeast Mexico. Samples were collected from late May to June and from late June to July (sampling year is unknown). Methoxychlor was detected in the blood of 6 out of 32 green turtle samples (18,75%) with concentrations

in the range of 2.46–45.86 ng/g lipid (mean: 17.78 ng/g lipid; median:14.06 ng/g lipid; SD:15.78). However, the substance was not detected in the eggs of green turtles (n=28). Methoxychlor was detected in the blood of 7 out of 28 hawksbill turtle samples (25%) with concentrations in the range of 1.96–38.34 ng/g lipid (mean: 12.43 ng/g lipid; median: 7 ng/g lipid; SD: 12.80). Methoxychlor was detected in the eggs of 4 out of 27 hawksbill turtle samples (14.81%) with concentrations in the range of 290.4–3564 ng/g lipid (mean: 1562 ng/g lipid; median: 1198 ng/g lipid; SD: 1407).

2.3.2 Human exposure

15. The 2013 pesticide monitoring results in the EU (including Iceland and Norway) (EFSA, 2015) summarised the results provided by the reporting countries and identified areas of concern regarding sample compliance with the legal limits of pesticide residue in foods. EFSA also assessed the consumer dietary exposure to pesticide residues in the sampled food commodities and performed an analysis of the chronic and acute dietary risks for European consumers. The outcome of this monitoring (EFSA, 2015) showed that methoxychlor was detected in milk and honey after it was banned in the EU in 2006. Methoxychlor was detected/quantified in 7 samples out of 1497 animal products. Maximum Residue Level (MRL) (0.01 mg/kg set at the LOQ (0.01 ppm) exceedances were noted for methoxychlor in animal products (fat of swine, bovine and poultry from Spain and Belgium, the range of measured residue levels: 0.018–0.021 (mg/kg)). Methoxychlor was found in concentrations above the LOQ, but the estimated dietary exposure was below the toxicological reference values (acceptable daily intake (ADI) of 0.1 mg/kg bw per day established by the Joint Meeting on Pesticide Residues (1977)). Based on the analysis of the 2016 pesticide monitoring results in the EU (including Iceland and Norway) (EFSA, 2018), methoxychlor was again quantified in honey, but non-detectable in other commodities. However, it was only detected above the LOQ in 2 out of 57141 food samples from 30 countries. Based on concentrations found for methoxychlor in the consumer products from the 2016 monitoring, it was concluded that the short- and long-term dietary exposures were unlikely to pose a health risk to EU consumers.

2.3.3 Exposure – comparison with POP substances

16. Lopez-Espinosa *et al.* (2008) investigated the presence of OCP residues in 52 fat samples of boys (mean age 7 years old (0–15 years old)) living in Southern Spain. The occupations and activities of the parents of the boys are unknown; however, the samples do provide information about the exposure to children in a predominantly agricultural area. According to the authors, children can be exposed to OCPs *in utero* via the placenta (Lopez-Espinosa *et al.*, 2007) and after birth via lactation (Noren and Meironyte, 2000; Solomon and Weiss, 2002). Fat samples were collected between 1994 and 1996, while methoxychlor was still in use in the EU. Methoxychlor was found in adipose tissues of 3 out of 52 children with a mean concentration of 16±20 ng/g lipid (Percentiles among ≥ LOD: P25=52 ng/g lipid; P50=121 ng/g lipid and P75=680 ng/g lipid). The mean concentration (±S.D.) was calculated for the whole group and 25, 50, and 75 percentiles were calculated for samples with methoxychlor concentrations ≥LOD. Raw data are not available, and it is unclear how data <LOD have been considered in the calculation of the mean concentration and if on which distribution was used for calculating the percentiles. Comparable concentrations in adipose tissues were found for Lindane (mean value of 11±12 ng/g lipid; Percentiles among ≥ LOD: P25 = 29 ng/g lipid; P50=71 ng/g lipid and P75=106 ng/g lipid) which was found in 6 out of 52 children. The exposure scenarios are not fully known, but the exposure of Lindane may have been greater. The study was conducted before its inclusion to the Stockholm Convention on POPs in 2009.

17. Cabrera-Rodriguez *et al.* (2020) found methoxychlor in 4 out of 447 (0.9%) umbilical cord blood samples from La Palma (Canary Islands, Spain) collected between March 2015 and April 2016 with laboratory recoveries ≥ 98.2% for methoxychlor (Pérez Luzardo personal communication, April 2020). Concentrations of methoxychlor in umbilical cord blood samples were in the range 0.004–0.132 ng/mL, while methoxychlor was banned in the EU in 2006. Similar levels have been found for aldrin (0.002–0.161 ng/mL; n>LOD = 13/447; it was banned in Europe in the early 1980s) and mirex (0.019–0.09 ng/mL; n>LOD = 9/447; it has never been authorised in Europe).

18. Jimenez Torres *et al.* (2006) determined the level of OCPs in the adipose tissue and serum of 72 women giving birth (aged from 18–35 years) in Southern Spain. The date of sampling is unknown. Methoxychlor was found in 3 out of 72 (4.1%) adipose tissue samples from pregnant women at a concentration range of 106–817.85 ng/g of fat (mean value: 347.73 ng/g of fat; SD: 407.19 ng/g of fat; recovery rate of 97%). However, methoxychlor was not detected in the serum. Of the samples with detectable levels, the mean concentration of methoxychlor in the fat of the women was higher than the mean concentration of the POP Lindane (mean:113.82 ng/g of fat; concentration range: 4.22–407.37 ng/g of fat detected in 36 out of 72 (50%) adipose tissue samples).

2.3.4 Information on bioavailability

19. Insufficient data is available to adequately define the bioavailability of methoxychlor (Office of Parliamentary Counsel of Canberra, 2013). However, it is expected that the high potential of adsorption (high log K_{oc} values) of methoxychlor to solids (sediments, soils and particulate matters) may reduce its bioavailability in sediment, soil and water compartments.

2.4 Hazard assessment for endpoints of concern

20. The European Chemicals Agency (ECHA) online Classification & Labelling (C&L) Inventory database[20], reports 115 notifications for methoxychlor as of 7 May 2020. 94 notifiers have classified methoxychlor as harmful if swallowed (Acute Toxicity Category 4, H302), may cause damage to organs (STOT SE 2, H371 (not specified)) and very toxic to aquatic life (Aquatic Acute Category 1, H400). 16 notifiers have classified methoxychlor as harmful if swallowed, in contact with skin or if inhaled (Acute Toxicity Category 4, H302, H312, H332); suspected of damaging fertility or the unborn child (Reprotoxic Category 2, H361), may cause damage to organs through prolonged or repeated exposure (STOT RE 2, H373 (eye, oral)), very toxic to aquatic life (Aquatic Acute Category 1, H400) and very toxic to aquatic life with long lasting effects (Aquatic Chronic Category 1, H410). Three notifiers indicated no classification according to the CLP criteria. One notifier has classified methoxychlor as harmful if swallowed, in contact with skin or if inhaled (Acute Toxicity Category 4, H302, H312, H332); suspected of causing cancer (Carcinogen Category 2, H351), very toxic to aquatic life (Aquatic Acute Category 1, H400) and very toxic to aquatic life with long lasting effects (Aquatic Chronic Category 1, H410). One notifier has classified methoxychlor as suspected of damaging fertility or the unborn child (Reprotoxic Category 2, H361); may cause damage to organs (STOT SE 2, H371 (nervous system)), may cause damage to organs through prolonged or repeated exposure (STOT RE 2, H373 (Liver, nervous…)), very toxic to aquatic life (Aquatic Acute Category 1, H400) and very toxic to aquatic life with long lasting effects (Aquatic Chronic Category 1, H410).

2.4.1 Ecotoxicological effects

2.4.1.1 Adverse effects on aquatic organisms

2.4.1.2 Adverse effects on terrestrial organisms

21. The primary metabolite of methoxychlor is 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl) ethane (HPTE) has been shown to alter viability and differentiation of embryonic thymocytes. Leung-Gurung *et al.* (2018) explored the impact of HPTE on a critical window and component of immune system development, embryonic T-cell development. Embryonic thymocytes (GD 16-18) from C57BL/6 mice were subjected to an *in vitro* differentiation culture that mimicked early steps in thymocyte development in the presence of 0.005, 0.05, 0.5, 5, or 50 µM HPTE, or diethylstilbestrol (DES; CAS No. 56-53-1). HPTE- and DES-induced death of thymocytes (Annexin-V and Caspase 8 were used markers of apoptosis). Moreover, HPTE-induced cell death not only resulted in selective loss of double positive thymocytes, but also loss of developing CD4 intermediate cells (post-double positive partially differentiated thymocyte population). Phenotypic analysis of thymocyte maturation (T-cell receptor, TCR) and TCR ligation (CD5) surface markers revealed that surviving embryonic thymocytes expressed low levels of both. Taken together these data demonstrate that immature embryonic thymocytes are sensitive to HPTE exposure and that HPTE exposure targets thymocyte populations undergoing critical differentiation steps. These findings suggest HPTE may play a pivotal role in metoxychlor exposure-induced immune dysfunction.

2.4.1.3 Summary of ecotoxicological effects

2.4.2 Adverse effects on human health

2.4.2.1 Epidemiological studies

2.4.2.2 Neurotoxicity

2.4.2.3 Endocrine disruption

22. One common mode of action by which endocrine disrupting chemicals, produce lasting reproductive tract defects is through persistent alteration of developmental gene expression. Fei *et al.* (2005) examined the uterine response to methoxychlor and its effect on *hoxa10* (a gene necessary for uterine development and function) expression in adult female and newborn mice. The authors also examined the effect of *in vitro* treatment on *HOXA10* gene expression in Ishikawa cells. Fei *et al.* (2005) found that methoxychlor treatment (1mg/day dissolved in DMSO; intraperitoneal (ip) injections) in mice suppressed the expression of *hoxa10*. The uterotrophic response to ip administration of methoxychlor (1 mg/day) was an increase in uterine wet weight of ca. 1.25-fold and a change in epithelial height. This suppression in HOXA10 expression (measured using immunohistochemistry (IHC)) was observed when mice were exposed neonatally (for 14 days to 2 mg/kg/day dissolved in DMSO; ip injections) and decreased expression persisted into early adulthood. It is unclear whether IHC intensity of HOXA10 correlates with total protein concentration. Immunoblotting would have provided more quantitative information. Also, it is possible that the diffusion of intensity is due to changes in cellular localization. The authors observed that *in vitro* exposure to methoxychlor induced *HOXA10* gene expression in Ishikawa cells (treated with methoxychlor at concentration ranging from 1 to 50 µM dissolved in DMSO). HOXA10 mRNA levels in Ishikawa cells (measured by RT-PCR) were increased 6- to 8-fold after treatment with 25 or 50 µM methoxychlor. In addition, treatment with 1 to 50 µM of methoxychlor treatment resulted in a dose-dependent increase in HOXA10 protein expression (quantified by western

[20] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/112624.

blot analysis). According to the authors (Fei *et al.,* 2005), the apparent contrast in the *in vitro* and *in vivo* effects is likely due in part to more efficient metabolism of methoxychlor in the mouse than *in vitro*. Hydroxy and bis-hydroxy metabolites of methoxychlor are more potent endocrine disruptors. Additionally, *in vitro* (in the absence of estradiol (E2)) methoxychlor functioned as a weak E2 agonist, whereas in the presence of E2, *in vivo* methoxychlor functioned as an E2 antagonist.

23. Zama and Uzumcu (2009) reported on studies looking at effects on rats during gestation and early stages post-natally. Based on doses of methoxychlor at 0.02 mg/kg/day, and 100 mg/kg/day between embryonic day 19 and postnatal day 7, the authors concluded that, even at the lower dose, methoxychlor exposure during fetal development caused epigenetic changes as a result of hypermethylation resulting in impaired function of ovaries in newborns. In particular, methoxychlor resulted in significant hypermethylation in the estrogen receptor 2 (ER2) promoter regions in the ovaries; thus potentially affecting the production and processing of estrogen.

2.4.2.4 Interaction with other chemicals

24. Methoxychlor has also been shown to interact with the phytoestrogen genistein to alter the toxicological effects of methoxychlor (Wang *et al.* (2006); You *et al.* (2002, 2006)). Exposure to genistein and methoxychlor during pregnancy resulted in significant feminization of male pup mammary glands. In male pups, there was prominent elongation of the glandular ducts, and development of an alveolar-lobular structure, which was not observed following exposure of either compound alone. Microarray analysis showed that these effects may be due to interactions involved in steroid signalling, growth factor pathways, apoptosis, and/or tissue remodelling. These results suggest that juvenile males may be more sensitive to endocrine-active compounds such as methoxychlor, and that phytoestrogens, such as genistein, may further modulate toxicity.

2.4.2.5 Summary of adverse effects on human health