PBT factsheet n°

Substance Name(s): Methoxychlor

EC Number(s): 200-779-9

CAS number(s): 72-43-5

SMILES: COc1ccc(cc1)C(c2ccc(OC)cc2)C(Cl)(Cl)Cl

Chemical formula (2D- structure): $C_{16}H_{15}Cl_3O_2$

Date: 24 May 2017

Substance prioritised for screening/assessment based on: a literature review of publications

SUMMARY OF DISCUSSIONS

DISCUSSION AT THE PBT MEETING 1 (DATE)	
UPDATE AFTER MEETING 1	

CONCLUSION

(Please tick where appropriate)			*(Remarks)*
☒	1.	**PBT/vPvB properties fulfilled**	Methoxychlor should be considered a P (persistent), vB (very Bioaccumulative) and T (toxic) substance. Furthermore, its probable potential for long range transport makes it eligible as a POP substance.
☐	2.	**Equivalent level of concern to PBT/vPvB**	
☐	3.	**Further testing/information needed on:**	☐ Compliance check / ☐ Substance evaluation
	☐	P:	
	☐	B:	
	☐	T:	
☐	4.	**Not PBT/vPvB**	
☐	5.	**Further discussion necessary**	

RESULTS OF EVALUATION OF PBT/VPVP PROPERTIES

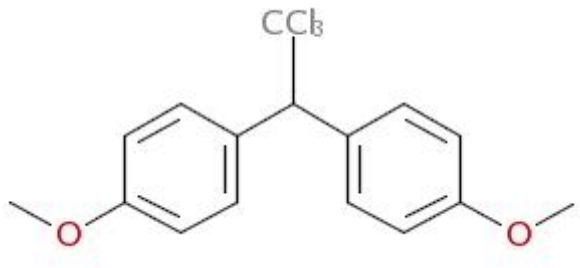
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	200-779-9
EC name:	Methoxychlor
SMILES	COc1ccc(cc1)C(c2ccc(OC)cc2)C(Cl)(Cl)Cl
CAS number (in the EC inventory):	72-43-5
CAS number:	72-43-5
CAS name:	Methoxychlor
IUPAC name:	1,1'-(2,2,2-Trichloroethylidene)bis(4-methoxybenzene)
Index number in Annex VI of the CLP Regulation	Not classified
Molecular formula:	$C_{16}H_{15}Cl_3O_2$
Molecular weight range:	345.65 g/mol
Synonyms:	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 Maralate Marlate para,para'-Methoxychlor Metox Methoxy-DDT 1,1,1-Trichloro-2,2-bis(para-methoxyphenyl)ethane 1,1,1-Trichloro-2,2-di(4-methoxyphenyl)ethane

Structural formula:



1.2 Composition of the substance

Name: Methoxychlor

Description: White to light-yellow crystals (Lewis, 2007; Lide, 2007; O'Neil *et al.*, 2006; Tomlin, 1997)

Degree of purity: Not available

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
Methoxychlor	Not available	Not available	-

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks
3,6,11,14-tetramethoxydibenzo(g,p)chrysene	Not available	Not available	-

Table 4: Additives

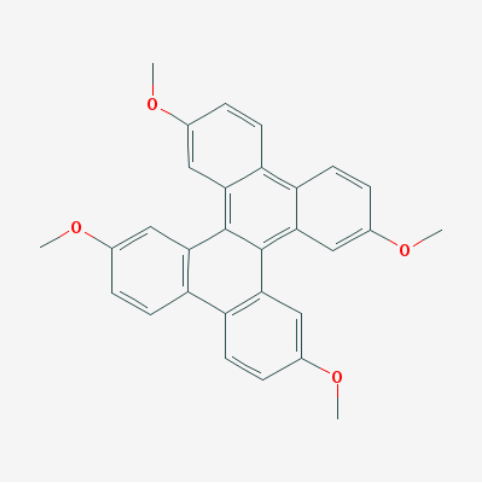
Additives	Typical concentration	Concentration range	Remarks
Not available	Not available	Not available	-

1.3 Identity and composition of degradation products/metabolites relevant for the PBT assessment

Table 5: Degradation (transformation) product/metabolite

EC number:	
EC name:	
SMILES:	COC1=CC2=C3C4=C(C=CC(=C4)OC)C5=C(C3=C6C=C(C=CC6=C2C=C1)OC)C=C(C=C5)OC
CAS number (in the EC inventory):	
CAS number:	60223-52-1
CAS name:	3,6,11,14-tetramethoxydibenzo[g,p]chrysene
IUPAC name:	3,6,11,14-tetramethoxydibenzo[g,p]chrysene
Index number in Annex VI of the CLP Regulation	
Molecular formula:	$C_{30}H_{24}O_4$
Molecular weight range:	448.50916 g/mol
Synonyms:	tetramethoxydibenzochrysene

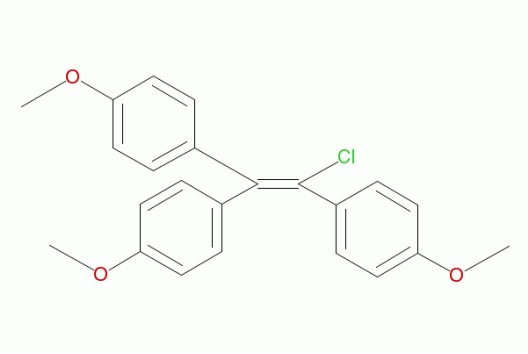
Structural formula:



Indication of the process, organism and/or organ in which the formation takes place: product of phototransformation in air of Methoxychlor

EC number:	209-318-6
EC name:	Chlorotrianisene
SMILES:	Cl/C(c1ccc(OC)cc1)=C(/c2ccc(OC)cc2)c3ccc(OC)cc3
CAS number (in the EC inventory):	569-57-3
CAS number:	569-57-3
CAS name:	Chlorotrianisene
IUPAC name:	1,1',1''-(2-chloroethene-1,1,2-triyl)tris(4-methoxybenzene)
Index number in Annex VI of the CLP Regulation	
Molecular formula:	$C_{23}H_{21}ClO_3$
Molecular weight range:	380.864 g/mol
Synonyms:	chlorotris(4-methoxyphenyl)ethylene

Structural formula:



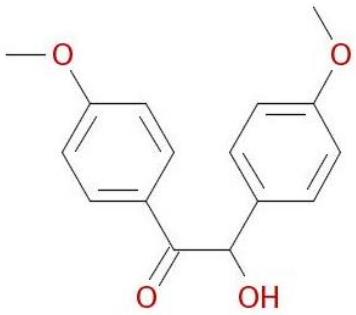
Indication of the process, organism and/or organ in which the formation takes place: product of phototransformation in air of Methoxychlor

Structural formula:

Indication of the process, organism and/or organ in which the formation takes place: product of in air of Methoxychlor

EC number:	204-330-8
EC name:	4,4'-anisoin
SMILES:	O=C(c(ccc(OC)c1)c1)C(O)c(ccc(OC)c2)c2
CAS number (in the EC inventory):	119-52-8
CAS number:	119-52-8
CAS name:	4,4’-anisoin
IUPAC name:	4,4’-anisoin
Index number in Annex VI of the CLP Regulation	Not listed
Molecular formula:	C16H16O4
Molecular weight range:	272.2958
Synonyms:	4,4'-dimethoxybenzoin

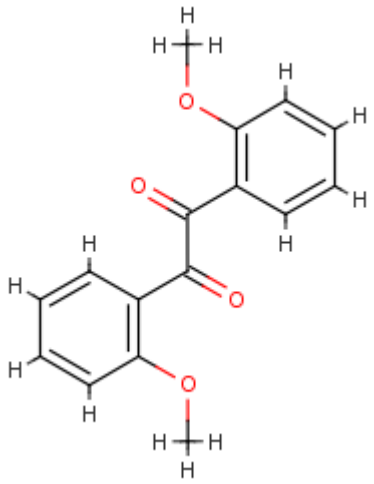
Structural formula:



Indication of the process, organism and/or organ in which the formation takes place: hydrolysis

EC number:	
EC name:	
SMILES:	COc1ccccc1C(=O)C(=O)c2ccccc2OC
CAS number (in the EC inventory):	6706-92-9
CAS number:	6706-92-9
CAS name:	o-anisil
IUPAC name:	
Index number in Annex VI of the CLP Regulation	Not listed
Molecular formula:	C16H14O4
Molecular weight range:	270.28
Synonyms:	

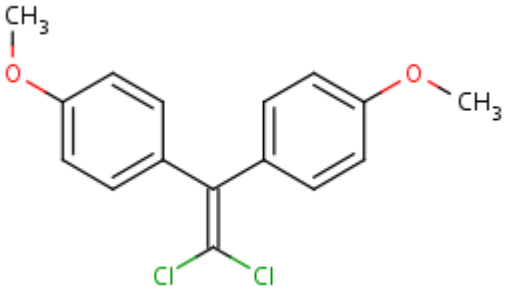
Structural formula:



Indication of the process, organism and/or organ in which the formation takes place: hydrolysis

EC number:	
EC name:	
SMILES:	COc1ccc(cc1)C(=C(Cl)Cl)c2ccc(cc2)OC
CAS number (in the EC inventory):	
CAS number:	2132-70-9
CAS name:	1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene
IUPAC name:	
Index number in Annex VI of the CLP Regulation	Not listed
Molecular formula:	C16H14Cl2O2
Molecular weight range:	309.187
Synonyms:	p,p'-Methoxychlor olefin

Structural formula:



Indication of the process, organism and/or organ in which the formation takes place: hydrolysis and photolysis in water

Other degradation products not clearly identified, such as substances produced by biodegradation in sediments: dechlorinated methoxychlor (DMDD) and mono- and di-hydroxy derivatives of methoxychlor and dechlorinated methoxychlor.

1.4 Identity and composition of structurally related substances (grouping approach)

Table 6: Structurally related substance(s) identity

EC number:	
EC name:	
SMILES:	
CAS number (in the EC inventory):	
CAS number:	
CAS name:	
IUPAC name:	
Index number in Annex VI of the CLP Regulation	
Molecular formula:	
Molecular weight range:	
Synonyms:	

Structurally related substance(s) formula:

Degree of purity:

Table 7: Constituents of structurally related substance(s)

Constituents	Typical concentration	Concentration range	Remarks
Name and EC number			

Table 8: Impurities of structurally related substance(s)

Impurities	Typical concentration	Concentration range	Remarks
Name and EC number			

Table 9: Additives of structurally related substance(s)

Additives	Typical concentration	Concentration range	Remarks
Name and EC number			

1.5 Physico-chemical properties

Table 10: Overview of physicochemical properties[1]

Property	Value	Remarks	References
Physical state at 20°C and 101.3 kPa	solid	-	-
Melting/freezing point	87°C	*Experimental*	Lide, 2007
	129.34°C	*QSAR*	US-EPA, 2011
Boiling point	346°C 377.87°C	*Experimental* *QSAR*	US-EPA, 2011 US-EPA, 2011
Vapour pressure	5.56 10^{-3} Pa	*QSAR*	US-EPA, 2011
Surface tension	Not available	-	
Water solubility	0.302 mg/l at 25°C	*QSAR*	US-EPA, 2011
	0.04 mg/l at 24°C	*Experimental*	Verschueren, 1996
	0.01 mg/l at 25°C	*Experimental*	Richardson and Miller, 1960
Partition coefficient n-octanol/water (log value) log Kow	5.08	*Experimental*	*Hansch et al., 1995*
	5.67	*QSAR*	US-EPA, 2011
Partition coefficient n-octanol/air (log value) Log K_{OA}	10.161	*QSAR*	US-EPA, 2011
Partition coefficient air/water (log value) Log K_{AW}	-5.081	*Experimental*	US-EPA, 2011
Dissociation constant	-	-	-

[1] The references of the values reported in Table 5 will be available in the technical dossier. In case references need to be included an additional column could be added manually to Table 5.

2 CLASSIFICATION AND LABELLING

2.1 Harmonised Classification in Annex VI of the CLP

The substance is not classified in the Annex VI to Regulation (EC) No 1272/2008.

2.2 Proposal for harmonised classification in Annex VI of the CLP

Not relevant

2.3 Self classification proposed by the registrant

There are 4 self classifications available from the ECHA website (ECHA, 2011) in the CLP inventory for methoxychlor[2] (4 notifications for 9 notifiers). They are the following:

Notification 1 (4 notifiers)

Related to human health

Acute Tox. 4

H302: Harmful if swallowed.

STOT SE 2: Specific target organ toxicity – single exposure

H371: May cause damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

Related to environment

Aquatic Acute 1

H400: Very toxic to aquatic life

Notification 2 (3 notifiers)

No classification

Notification 3 (1 notifier)

Related to human health

Acute Tox. 4

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

H302, H312, H332: Harmful if swallowed, in contact with skin or if inhaled.

Carc. 2

H351 Suspected of causing cancer

Related to environment

Aquatic Acute 1

H400: Very toxic to aquatic life

Aquatic Chronic 1

H410: Very toxic to aquatic life with long lasting effects

Notification 4 (1 notifier)

Related to human health

Repr. 2

H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard).

STOT SE 2: Specific target organ toxicity – single exposure

H371: May cause damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

STOT RE 2: Specific target organ toxicity – repeated exposure

H373: May cause damage to organs (state all organs affected, if known) through prolonged or repeated exposure (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

Related to environment

Aquatic Acute 1

H400: Very toxic to aquatic life

Aquatic Chronic 1

H410: Very toxic to aquatic life with long lasting effects

3 ENVIRONMENTAL FATE PROPERTIES

3.1 Degradation

3.1.1 Abiotic degradation

Half-lives of methoxychlor in distilled water at 27°C are 1 year (pH 7), 5.5 years (pH 9), 0.21 days (pH 13) (Katagi, 2002).

The half-lives for methoxychlor in distilled water, and from a glass surface in winter, have been reported as 999.99 and 26.66 days (degradation rate of 0.03 1/day), respectively (Okamura, 1991).

Degradation of methoxychlor is slow in water. Half-life at pH 5-6 and 27°C is 100 days (Menzie, 1978). Methoxychlor applied in a sediment-water model rapidly degraded with a half-life of 4 to 7 days; the primary breakdown step of methoxychlor was due to the abiotic/microbial formation of reductively dechlorinated methoxychlor (Masuda, *et al*., 2012).

Methoxychlor is expected to be resistant to oxidation in water (Wolfe *et al.*, 1977; Zepp *et al.*, 1976).

3.1.1.1 Hydrolysis

The effect of hydrolysis and oxidation on the persistence of methoxychlor in the environment is expected to be negligible.

The hydrolysis half-life of methoxychlor in water at 27°C and pH 3-7 is estimated to be 367 days based on a rate constant of 2.2 10^{-8} /s (extrapolated from data at elevated temperatures) (Wolfe et al., 1977). At pH 9 its hydrolysis half-life has been estimated to be 270 days, although the hydrolysis half-life in soil is probably longer due to adsorption to the soil (Park, 1982).

At pH 7, anisoin and anisil are the major hydrolysis products of methoxychlor; 1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene (DMDE) is a minor product. At pH 10, 1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene is the major hydrolysis product of methoxychlor (Wolfe *et al.*, 1977).

3.1.1.2 Oxidation

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 *Phototransformation in air*

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).

If released to air, an estimated vapor pressure of 4.2 x 10^{-5} mm Hg at 25 °C indicates methoxychlor will exist in both the vapor and particulate phases (Bidleman, 1988). Vapor-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 photochemically-produced hydroxyl radicals has been estimated as 5.4 $10^{-11}cm^3$/mol-sec at 25°C (QSAR). This corresponds to an atmospheric half-life of about 7h at an atmospheric concentration of 5 x 10^5 hydroxyl radicals per cm^3 (Meylan and Howard, 1993).

Technical grade Methoxychlor contains 0.5% reactive hydrocarbon that is photo-oxidized in sunlight to dianisyl-dimethoxyphenanthrene that undergoes further reaction to form a weakly mutagenic tetramethoxydibenzochrysene. About 100 ppm of chlorotrianisene, a potent proestrogen which resembles diethylstilbestrol in structure and activity, is also present (Mitchell and West, 1979).

QSAR model estimation (AOPWin v4.10) predicts low persistence with a DT_{50} of 2.397 hours.

Particulate-phase methoxychlor may be removed from the air by wet and dry deposition.

3.1.1.3.2 *Phototransformation in water*

Assuming first-order kinetics, the midsummer photolysis half-life of methoxychlor is estimated to be 4.5 months in distilled water at latitude 40°N, and 2.2-5.4 hours in filtered, sterilized, natural waters at latitude 34°N (Zepp *et al.*, 1976).

According to some other authors, photolytic half-life in distilled water is 37 days. In some river waters, methoxychlor photolysis in sunlight is rapid with half-life of 2-6 hours. Ethylene analogue (DMDE) is formed in each case (Menzie, 1978).

DDE and 1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene, degradation products of DDT and methoxychlor, rapidly undergo an unusual photoisomerization in solution when exposed to sunlight; the isomerization involves the exchange of a vinyl chlorine and an ortho aromatic hydrogen (other photoproducts identified were corresponding benzophenones and 1,1-diaryl-2-chloroethylenes) (Zepp *et al.*, 1977). In distilled water, 1,1-bis(p-methoxyphenyl)- 2,2-dichloroethylene is the major photolysis product of methoxychlor and p-methoxybenzaldehyde is a minor product (Zepp *et al.*, 1976). The UV irradiation of methoxychlor in the presence of methyl oleate produced several addition products in a reaction performed to mimic the photoinduced addition of pesticides to biomolecules (Schwack, 1988). The half-lives for photodecomposition of methoxychlor (40 ppb) under sunlight in distilled water (pH 6.3), Suwannee river (pH 4.7), Tombigbee river (pH 7.6), Alabama river (pH 7.7), and 20 ppm "humic acid" in distilled water (pH 5.2) were 300, 2.2, 5.4, 2.9, and 7.3 hours, respectively, and there was no reaction in 2 hours for Withlacoochee river (pH 8.2) and South Georgia stream (pH 7.2) (Sundstrom and Ruzo, 1978).

3.1.1.3.3 *Phototransformation in soil*

Methoxychlor may undergo photolysis on soil surfaces based on studies reporting the photolysis of dry methoxychlor films exposed to sunlight (see section 3.1.1.3.1).

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

First-order rate constants of 0.001 to 0.004 (half-life of 7 to 29 days), 0.002 to 0.003 (half-life of 9.6 to 14.4 days), 0.001 to 0.006 (half-life of 4.8 to 29 days), and 0.002 to 0.004 per hour (half-life of 7 to 14.4 days) were measured for methoxychlor added to 4 different freshwaters (Paris and Rogers, 1986).

In an aerobic die-away study using water from the Santa Rosa Sound (Florida), methoxychlor had a half-life of >25 days (Walker *et al.*, 1988). In an aerobic shake-flask river die-away test, methoxychlor was biodegraded in a mixed water/sediment sample, rate not given (Cripe *et al.*, 1987).

Under anaerobic conditions (incubation time 118h), approximately 75% of a 200 µg sample of analytical grade MeO-DDT (acetone vehicle) was degraded to MeO-DDE by *Aerobacter aerogenes*. However, under aerobic conditions only 10% of the MeO-DDT was degraded to MeO-DDE (Mendel *et al.*, 1967).

Various microaerobic-facultative anaerobic bacteria originating in the soil-water environment have been shown to rapidly mediate the reductive dechlorination of methoxychlor under weak, slightly reductive conditions (Satsuma and Masuda, 2012). These findings suggest that methoxychlor is transformed to dechlorinated methoxychlor relatively easily in a submerged, surface water environment.

3.1.2.1.1 *Estimated data*

QSAR model estimation (BioWin v4.10) predicts persistence over weeks for methoxychlor.

3.1.2.1.2 *Screening tests*

3.1.2.1.3 *Simulation tests*

3.1.2.2 Biodegradation in sediments

Second-order rate constants of 5.2 10^{-14} and 6.1 10^{-16} l/organism/h (half-lives of 111 and 9500 days, respectively, assuming a bacterial concentration of 5 10^{9} organisms/l) were measured dependent on 15% and 90% sorption, respectively, to sediments in river die-away studies (Baughman and et al., 1980).

The degradation of C^{14}-methoxychlor was studied under laboratory conditions in lake and pond sediments (water-sediment ratio, 10-20:1) incubated under various redox conditions at 22.5°C or 10°C (Muir and Yarechewski, 1984). It was observed that methoxychlor added to sediment-water mixtures was slowly biodegraded under aerobic conditions (half-lives of 115 and 206 days) and more rapidly degraded anaerobically with half-lives of <28 days. The major degradation products were dechlorinated methoxychlor (DMDD) and mono- and di-hydroxy derivatives of methoxychlor and dechlorinated methoxychlor. Dihydroxy- derivatives represented 15% of extractable radioactivity in pond sediments and 28% in lake sediments after 448 days of aerobic incubation. Under N aeration, mono- and dihydroxy- derivatives represented 44 to 65% of extractable ^{14}C after 28 days incubation.

Biodegradation of methoxychlor by native strains of Streptomyces bacteria isolated from pesticide-contaminated sediments has also been reported (Bourguignon et al., 2014). This actinobacterium was able to remove the pesticide, reaching its maximum removal percentages (40% and 76%) after 28 days of incubation. Methoxychlor was gradually converted into 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane, 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethylene, 1-chloro-2,2-bis(4-methoxyphenyl)ethane, and 2,2-bis(4-hidroxyphenyl)acetonitrile, indicating that methoxychlor is dominantly degraded by dechlorination, dehydrogenation and CN-replacement, resulting in the production of several major degradation products.

In a study isolating a bacterium that mediates oxidative transformation of methoxychlor from river sediment, the O-demethylation of methoxychlor yielded a monophenolic derivative [mono-OH;

1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane] as the primary degradation product (Satsuma *et al*., 2012).

3.1.2.3 Biodegradation in soil

The degradation of methoxychlor in 2 sandy loam soils was dependent on water content; soils with 3% and 10% water content degraded methoxychlor to only trace amounts within 30 to 38 and 20 to 26 weeks, respectively (Obuchowska, 1969). Methoxychlor applied to soil during a 2-year field study was fairly persistent; residues remaining after 1, 2, and 3 months made up 92, 38, and 27% of the original application (Golovleva *et al.*, 1984). One year later, measurable concentrations of methoxychlor were still present in soil as well as products from the partial dechlorination of the parent compound (Golovleva *et al.*, 1984).

Methoxychlor was incubated in soil under aerobic and anaerobic conditions (Fogel *et al*., 1982). Primary degradation of methoxychlor occurred under anaerobic conditions, but not under aerobic conditions after 3 months of incubation. When anaerobically incubated methoxychlor was subsequently transferred to an aerobic environment, it underwent biodegradation at a rate which was as much as 70-fold greater than that of methoxychlor exposed solely to aerobic incubation. This suggests a cometabolic processes may be responsible for the extensive molecular changes which occurred with methoxychlor because the rate of its disappearance from soil was observed to level off after exhaustion of soil organic matter. After this incubation period, soils previously incubated under anaerobic conditions were converted to aerobic conditions, suggesting the initial dechlorination step renders the chemicals more susceptible to further aerobic biodegradation. (Fogel *et al*., 1982).

Anaerobic biodegradation of methoxychlor resembles that of DDT – proceeding primarily by dechlorination to form DMDD, analogically to DDT conversion to DDD (Fogel et al., 1982). Also mono- and dihydroxy (demethylated) derivatives of methoxychlor and DMDD are formed (Muir and Yarechewski, 1984).

Species of white rot fungi Phanerochaete chrysosporium (Grifoll and Hammel, 1997) and Stereum hirsutum (Lee *et al*., 2007) have been shown to mineralise and metabolise ^{14}C-methoxychlor. A variety of dechlorination and dehydrogenation degradation products including 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane, 2,2,2-trichloro-1,1-bis(4 methoxyphenyl)ethanol, 2,2-dichloro-1,1-bis(4-methoxyphenyl)ethanol, 2,2-dichloro-1,1-bis(4-methoxyphenyl)ethane, 2,2-dichloro-1,1-bis(4-methoxyphenyl)ethylene, 2-chloro-1,1-bis(4-methoxyphenyl) ethane, 2-chloro-1,1-bis(4-methoxyphenyl) ethylene, and 1,1-bis(4-methoxyphenyl)ethylene were identified.

Anaerobic biodegradation of methoxychlor in field polluted soil was tested at 12, 22 and 30°C using methanogenic granular sludge as inoculum (Baczynski *et al*., 2009). Temperature was shown to have a relatively small impact on methoxychor degradation in this process. In the first 2 weeks its concentration decreased by 90% at 30 °C, temperature lowering to 12°C reduced removal efficiency to 78% (Baczynski *et al*., 2009).

3.1.2.4 Summary and discussion on biodegradation

Biodegradation data for methoxychlor in water are weak and half-life is unknown (only indication is >25days). QSAR model estimation predicts persistence over weeks for methoxychlor in water.

In sediments, methoxychlor half-life varies between 28 to 206 days depending on aeration intensity. Finally, in soils, methoxychlor degrades within weeks (20 to 38 weeks).

In conclusion, biodegradation data available in sediments and soils are sufficient for methoxychlor to be considered persistent (P) in the environment.

Biodegradation of methoxychlor in soils and sediments is complex but evidence suggests the process is more rapid in anaerobic conditions and less rapid under aerobic conditions.

3.1.3 Summary and discussion on degradation

According to experimental data, the hydrolysis half-life of methoxychlor in water varies between 270 to 367 days and hydrolysis is therefore not a significant abiotic degradation process of this substance. As regards phototransformation in air, data seem to indicate a rapid degradation in this media, while for phototransformation in water results are not conclusive. Finally, methoxychlor seems to biodegrade rather slowly in the environment but main information available allow to conclude on its persistency.

According to estimated data, degradation of methoxychlor may be rather low, lasting weeks. Half-lives in sediment-water system under aerobic conditions exceed the Annex D POP Criteria.

In summary, main information available for methoxychlor allow to conclude on its persistency in the aquatic environment and in soils while its photo-oxidation with hydroxyl radicals is fast. Methoxychlor being degraded in anisoin, anisil, and DMDE (proportion depending on pH), these metabolites should be also considered for their PBT properties. Furthermore, phenolic metabolites excreted by animals mainly via feces should be considered for their persistency as well given their potential for reproductive and developmental effects (cf. section 4.1.3).

3.2 Environmental distribution

3.2.1 Adsorption/desorption

Measured average sorption coefficient (Koc) values in various 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). In another study, a K value of 620 was found in a water-sediment system (Wolfe *et al.*, 1977). Another study determined a K value of 2009 in soil (Weber *et al.*, 2004). According to a classification scheme (Swann *et al.*, 1983), these Koc values suggest that methoxychlor is expected to be immobile in soil. Mass balances in pond, river and wet sand (25% moisture) systems spiked with methoxychlor indicate that 98.6, 97.0, and 92.1%, respectively, of all methoxychlor was retained in the sediment (Muir *et al.*, 1983).

3.2.2 Volatilisation

The Henry's Law constant for methoxychlor is $2.03\ 10^{-7}$ atm.m^3/mol (Altschuh *et al.*, 1999). This Henry's Law constant indicates that methoxychlor is expected to be essentially nonvolatile from water (Lyman *et al.*, 1990). Based upon vapour pressure estimated by QSAR, Methoxychlor is not expected to volatilize from dry soil surfaces, determined from a fragment constant method (Lyman, 1985).

An octanol–air partition coefficient (K_{OA}) has been derived for methoxychlor (Odabasia and Cetin, 2012). The Log K_{OA} value for methoxychlor at 25 °C was estimated to be 10.48. This value was used to derive net soil–air gas exchange fluxes, which suggest volatilization fluxes are generally much lower than the sum of input fluxes (dry deposition, wet deposition and gas absorption) indicating a net deposition of methoxychor to soils (Odabasia and Cetin, 2012).

3.2.3 Distribution modelling

3.3 Potential for long range transport

According to its physico-chemical properties (see section 1.5), methoxychlor might not be considered as a candidate for Long Range Transport (LRT). This could be considered confirmed by its "characteristic travel distances" (CTD) constant of 55 km very low compared to some other current use pesticides (e.g. lindane with a CTD of 2500 km or pentachloronitrobenzene with a CTD of 12,100 km) as calculated in an AMAP publication (Hoferkamp *et al.*, 2010).

OECD LRT model (Wegmann *et al.*, 2009) was also runned to attempt assessing the potential for LRT of methoxychlor. Screening results as available in the Table below confirm a potential for LRTP with overall persistence (P_{OV}) value estimated at 526 days in the environment and Characteristic Transfer Distances (CTD) values ranging between 792 and 849 km depending on octanol-water partition coefficient. In fact, methoxychlor does not reach the high P_{OV} of PCB 180 (5.10^4 days), nor the high LRTP of carbon tetrachloride (CTD of 1.2 10^6 km), but it exhibits P_{OV}, CTD values not far from some identified POPs (e.g. "aldrin-21" presents a POV of 688 days while "biphenyl-7" shows a CTD of 423 km). However, methoxychlor estimated Transport Efficiency of 0.005 – 0.008 % is very low in all cases. This estimation is based on the very fast atmospheric degradation of the substance, which makes the LRTP questionable

Screening methoxychlor overall persistence (P_{OV}) and long range transport potential (LRTP) by running the OECD software tool (Wegmann *et al.*, 2009).

Chemical properties used as input data for calculations of P_{OV} and LRTP	
log K_{AW}	-5.081
log K_{OW}	5.08 – 5.67
Half-life in air (hours)	**2.4 hours**
Half-life in water (hours)	**ca. 365 days, that is 8760 hours**
Half-life in soil (hours)	**ca. 365 days, that is 8760 hours**

Screening results / outputs: P_{OV} and LRTP indicator values

Values are calculated for each environmental compartment (air, soil, water) and the most critical emission scenario is chosen, i.e. the maximum value (worst case) is systematically reported as the one to retain for the chemical assessed.

P_{OV} (days)	**526**
Critical emission scenario	***soil***
CTD (km)	**849 – 792**
Critical emission scenario	***water***
Transfer Efficiency (%)	**0.005 – 0.008**

Critical emission air scenario

In a matter of monitoring, methoxychlor has been measured at Arctic monitoring stations (Su *et al*., 2008) and has been detected in various media in the Arctic, including in air (e.g. Halsall *et al*., 1998; Hung *et al*., 2005), snow (e.g. Boyd-Boland *et al.*, 1996; Franz *et al*., 1997; Welch *et al*., 1991), ice core (e.g. Boyd-Boland *et al.*, 1996; Hermanson *et al.*, 2005) and in terrestrial (caribou, muskox and lamb), avian (kittiwake and thick-billed murre) and marine (snow crab and other invertebrates, fish, seals, beluga) biota samples (e.g. Vorkamp *et al*., 2004; Savinova *et al*., 2011). According to experts, the apparent atmospheric persistence of methoxychlor as shown by its occurrence in non-antropologically influenced regions is likely to result from little or no oxidation by OH during the dark polar winter and in spring. Given these observations, LRT of methoxychlor to pristine regions shall not be ruled out and should be studied in details.

Methoxychlor has also been measured in elephant seal (*Mirounga leonina*) pups on Elephant Island in Antarctica (Filho *et al*., 2009). The concentration of methoxychlor in samples collected from 7 dam/pup pairs of southern elephant seals were: 2.91 ± 1.17 ng g^{-1} lipid in Dam blubber, 1.79 ± 0.32 ng g^{-1} lipid in Milk and 1.86 ± 0.40 ng g^{-1} lipid in Pup blubber. The authors concluded that methoxychlor as a contaminant in Antarctica may reflect contamination from its potential current or historical use for agriculture purposes in the southern hemisphere.

As this species is resident to this location (i.e. not a migratory species) this observation indicates long range transport from sources in the southern hemisphere, where use of these insecticides in developing countries may still occur.

As a conclusion, even though models tend to estimate methoxychlor LRTP as low, measured levels of methoxychlor in locations distant from its sources of its releases indicate that long range transport occurred in certain atmospheric conditions were photodegradation is less likely.

Methoxychlor is therefore expected to meet the criteria for classification in terms of its long range transport to high latitude regions in both hemispheres.

3.4 Bioaccumulation

BCFWIN (EPIWIN v4.1) predicts a BFC QSAR of 1044 and an experimental BCF of 70 700 is reported in EPIWIN database, implying a very high bioaccumulative potential.

3.4.1 Bioaccumulation in aquatic organisms (pelagic and sediment organisms)

Bioconcentration factors (BCF) for methoxychlor as high as 8 300 in fathead minnow (*Pimephales promelas*) (Veith *et al.*, 1979) has been measured in continuous flow systems. These BCF values suggest that bioconcentration in aquatic organisms is very high, even if some variation between species in the ability to metabolize this compound may exist (Metcalf *et al.*, 1971). Bioconcentration factors (BCF) for methoxychlor of 12 000 in mussel (Renberg *et al.*, 1985), 5000-8570 in snail (*Physa integra*), 348-1130 in stonefly (*Pteronarcys dorsata*) (Anderson and DeFoe, 1980) and 1500 in soft clams (*Mya avenaria*)(Hawker and Connell, 1986) have also been measured in continuous flow systems.

Methoxychlor is readily metabolized to mono- and dihydroxy derivatives by fish and the parent compound stored in relatively low concentrations compared to other chlorinated pesticides (e.g. DDT) in a model terrestrial-water ecosystem (Metcalf *et al.*, 1971).

3.4.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

Methoxychlor is readily metabolized to mono- and dihydroxy derivatives by mosquito larvae and snails and the parent compound stored in relatively low concentrations compared to other chlorinated pesticides (e.g. DDT) in a model terrestrial-water ecosystem (Metcalf *et al.*, 1971).

3.4.3 Summary and discussion of bioaccumulation

QSAR models predicts a BCF of 1044 and an experimental BCF of 70 700 is reported in EPIWIN database, implying a very high bioaccumulative potential. Even if some variations between species in the ability to metabolize this compound may exist, according to data available in the literature (BCF of 8 300), methoxychlor should be considered a very Bioaccumulative substance (vB).

4 HUMAN HEALTH HAZARD ASSESSMENT RELEVANT FOR THE PBT ASSESSMENT

Information below was mainly retrieved from ATSDR (2002) and HSDB (2012).

Mechanism of action

Within the reproductive system, estrogenic stimulation of uterine and pituitary tissue typically causes a proliferative response accompanied by an angiogenic induction of new blood vessels from existing ones, thereby providing nutrients and oxygen to the growing tissue. The prooestrogenic pesticide methoxychlor, however, has shown a differential effect on proliferative activity. An increase in uterine growth is present, while the pituitary undergoes a decrease in size, even though the effect is accompanied by a characteristic estrogen-induced elevation in pituitary prolactin concentration (Goldman *et al.*, 2004).

Methoxychlor is a pesticide that has adverse effects on reproductive capability in mice. methoxychlor and its metabolites bind the estrogen receptor and function as endocrine disruptors. Methoxychlor diminishes the uterine decidual cell response, necessary for the support of pregnancy. Hoxa10 is an estrogen-regulated gene that is an essential mediator of the decidual response and necessary for pregnancy. A mechanism by which methoxychlor disrupts uterine function is by suppressing Hoxa10 expression (Fei *et al.*, 2005).

It has been suggested that methoxychlor acts through a different, as yet unelucidated, mechanism, not necessarily via an estrogen receptor (ER). Researchers showed that methoxychlor induced an increase in mRNA of two estrogen-responsive genes (lactoferrin, LF, and glucose-6-phosphate dehydrogenase, G6PD) in uteri of ovariectomized wild-type and ovariectomized ER(alpha)-knockout mice. Induction of LF and G6PD mRNA by methoxychlor was slightly greater in wildtype mice than in ER(alpha)-knockout mice at 15-30 mg/kg/day. Since no functional ER(alpha) receptors were present in the ER(alpha) knockout mice, the involvement of ER(beta) was indicated. However, when an estrogen inhibitor (ICI 182,780) was added, which should have eliminated methoxychlor interaction with ER(beta), methoxychlor still induced an increase in LF and G6PD mRNA at a slightly lower level than without inhibitor in wild-type mice and at the same level in ER(alpha) knockout mice. In the same experiment, estradiol-17(beta) resulted in large increases in LF and G6PD mRNA in wild-type mice, but in low levels of LF and G6PD mRNA (similar to control mice) in ER(alpha)-knockout mice (the authors point out that the concentration of ER(beta) in uterus of the wild-type and ER(alpha)-knockout mice was very low). When an inhibitor was added, no increase in LF and G6PD mRNA was induced by estradiol-17(beta) in wild-type mice. Thus, the "estrogenic" activity of methoxychlor was not attenuated by the absence of functional ER(alpha) receptors or the presence of an estrogen inhibitor effective at ER(beta), suggesting that an additional mechanism of toxicity may exist besides interaction with ER(alpha) or ER(beta) (ATSDR, 2002).

A study examined the effects of the xenoestrogen methoxychlor on messenger RNA (mRNA) concentrations of two estrogen-responsive uterine genes, lactoferrin (LF) and glucose-6-phosphate dehydrogenase (G6PD). Ovariectomized wild-type (WT) and estrogen receptor (ER)alpha-knockout (ERalphaKO) mice were treated with methoxychlor or estradiol-17beta (E2) to determine whether methoxychlor acts via pathways that involve ERalpha. In wild-type mice, both E2 and methoxychlor stimulated increases in uterine LF and glucose-6-phosphate dehydrogenase messenger RNA concentrations in a dose-dependent manner. Competitive pretreatment with the pure antiestrogen ICI 182,780 dramatically reduced E2-stimulated increases in mRNA concentrations but had no effect on methoxychlor-induced effects. Competitive pretreatment with E2 had only a partially inhibitory effect

on methoxychlor-induced responses. In the ERalphaKO mouse, E2 had little effect on uterine LF or G6PD mRNA concentrations, whereas methoxychlor stimulated marked increases in both LF and glucose-6-phosphate dehydrogenase messenger RNAs. The methoxychlor-induced increases in LF and glucose-6-phosphate dehydrogenase messenger RNAs in the ERalphaKO mouse were not suppressed by competitive pretreatment with either E2 or ICI 182,780. Fold increases in messenger RNA concentrations for both genes induced by methoxychlor were similar for wild-type and ERalphaKO mice. The results surprisingly indicate that a xenoestrogen, methoxychlor, can increase LF and glucose-6-phosphate dehydrogenase messenger RNA concentrations by a mechanism that is not mediated through ERalpha or ERbeta, and acts through another pathway (Ghosh *et al.*, 1999).

Investigators have elucidated the human cytochrome P450 enzymes responsible for conversion of methoxychlor into its major metabolite, the mono-O-demethylated derivative (mono-OH-M) that is estrogenic. Incubation of methoxychlor with microsomes from insect cells overexpressing either CYP1A2, CYP2C18, or CYP2C19 yielded mono-O-demethylated derivative with turnover numbers of 14.9, 15.5, and 39.1 nmol/min/nmol of P450, respectively. CYP2B6 and CYP2C9 were much less active. Incubations with purified CYP2C19 and CYP2C18 resulted in formation of mono-O-demethylated derivative, and also the bis-demethylated metabolite. Co-incubation of liver microsomes with methoxychlor and various P450 isoform-selective inhibitors suggested involvement of several P450s in mono-O-demethylation, including CYP1A2, CYP2A6, CYP2C9, and CYP2C19. A role for CYP2C19, CYP1A2, and CYP2A6 was also indicated by multivariate regression analysis of the mono-O-demethylase activity in a panel of human liver microsomes characterized for isoform-specific catalytic activities (R2 = 0.96). Based on the totality of the evidence, CYP2C19 appears to be the major catalyst of methoxychlor mono-O-demethylation. However, in individuals lacking functional CYP2C19 (eg the "poor metabolizer" phenotype), CYP1A2 may play the predominant role. CYP2A6, CYP2C9, and CYP2B6 probably contribute to a lesser extent. Although CYP2C18 is an efficient methoxychlor demethylase, its expression in liver is reportedly low or absent, suggesting a negligible role for this enzyme in methoxychlor metabolism. Lengthy incubations of liver microsomes with methoxychlor produced other secondary and tertiary metabolites (Stresser and Kupfer, 1998).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption

No studies were located regarding absorption of methoxychlor in animals after inhalation exposure (ATSDR, 2002).

Observations of adverse health effects in animals following oral exposure provide indirect evidence that ingested methoxychlor is absorbed by the gastrointestinal tract. Data from studies examining fecal and urinary excretion of radioactivity after oral administration of radiolabeled methoxychlor to mice and goats indicate that methoxychlor is rapidly and efficiently absorbed by the gastrointestinal tract (ATSDR, 2002).

In mice administered single doses of 50 mg/kg recrystallized, radiolabeled methoxychlor in oil by gavage, 90% of the dose was excreted as metabolites in the feces (85% polar metabolites) and 10% was excreted in urine (63.8% polar metabolites) within 48 hours (Kapoor *et al.*, 1970). Assuming that the fecal metabolites (primarily demethylated, dechlorinated, and dehydrochlorinated compounds) resulted from biliary excretion of absorbed material and not from degradation of unabsorbed parent by enteric bacteria, gastrointestinal absorption of methoxychlor appears to exceed 90% in mice.

In two lactating female goats administered single oral doses of 3.6 or 11.6 mg/kg laboratory grade, radiolabeled methoxychlor encapsulated in gelatin, 40.5 and 67.5% of the doses were excreted in the feces within 3 days, respectively, and 58.4 and 27.1% were excreted in the urine, respectively (Davison *et al.*, 1982). Metabolites (demethylated, dechlorinated, and dehydrochlorinated products of methoxychlor) were estimated to represent 70 and 81% of radioactivity in the feces, respectively. Assuming that metabolites in feces resulted from biliary excretion of absorbed material (and that the remaining radioactivity in feces was not absorbed), the data indicate that at least 87 and 82% of the respective administered doses were absorbed. In a bile-cannulated, castrated male goat given an oral dose of 25.6 mg/kg radiolabeled methoxychlor, 7.8, 35.2, and 44.4% of the radioactivity was excreted within 3 days in the bile, feces, and urine, respectively (Davison *et al.*, 1983). The profile of metabolites in the collected bile was reported to be similar to the metabolite profile in the feces collected from the female goats, but Davison et al. (1983) did not report any chemical analysis of the feces collected from the bilecannulated goat. The data provide support that methoxychlor is rapidly and efficiently absorbed by the mammalian gastrointestinal tract. However, interpretation of the goat studies is limited, because only one animal was tested per dose, bile cannulation may have influenced absorption, and ruminant toxicokinetic properties are not always relevant to nonruminant mammals.

Two studies in animals suggest dermal absorption of methoxychlor may range from 5 to 20%. In one study, a single dermal dose of 200 mg laboratory grade methoxychlor in dichloromethane was applied to the shaved backs of two goats. Three days later, 5–8% of the dose was recovered in the carcass, urine, and feces (Davison *et al.*, 1983). In the second study, four cows were dermally exposed to a single dose of 5 g methoxychlor in an emulsion (Skaare *et al.*, 1982). The levels of methoxychlor detected in milk were comparable to the levels in milk from cows given an intravenous dose of 1 g methoxychlor. The authors concluded that approximately 20% of the dermal dose was absorbed. Because of differences in skin, dermal absorption by goats and cows may not be a good model for dermal absorption by humans.

Distribution

No studies were located regarding distribution in animals after inhalation exposure to methoxychlor (ATSDR, 2002).

In animals, methoxychlor preferentially distributes to the fat but does not appear to persist. Furthermore, metabolic adaptation may enhance elimination. In rats exposed to 1.25, 5, or 25 mg/kg/day methoxychlor for 4–18 weeks, fat levels of methoxychlor were reported to be nondetectable, 1–7 mg/kg, and 14–36 mg/kg, respectively (Kunze *et al.*, 1950). Levels of methoxychlor in fat peaked during the first 9 weeks of exposure, after which time a gradual decline was noted during the last 9 weeks of exposure (Kunze *et al.*, 1950). Methoxychlor was not detected in the fat following the 2-week recovery period. In female rats exposed to 50, 125, or 250 mg/kg/day in the feed for 6 weeks, the levels of methoxychlor detected in abdominal fat were 21, 68, and 61 mg/kg, respectively (Harris *et al.*, 1974). The levels of methoxychlor in fat were 34 and 140 mg/kg in female rats when exposure to 50 and 125 mg/kg/day was continued through pregnancy and weaning (exposure duration=12 weeks) (Harris *et al.*, 1974). The authors speculated that the higher levels of methoxychlor in fat of female rats after pregnancy and weaning were due to a lower fat content in these animals. However, exposure duration (12 versus 6 weeks) may have been a factor as well. Levels of methoxychlor in the fat of sheep exposed to 6 and 49 mg/kg/day for 18 weeks peaked at 7.8 mg/kg by week 10, and at 24 mg/kg by week 6, respectively (Reynolds *et al.*, 1976). A steady decline of methoxychlor in fat was noted after week 10 in the low dose group, suggesting that metabolic adaptation enhanced elimination (Reynolds *et al.*, 1976). Methoxychlor was not detected in fat from either group following a 12–14-week recovery period. In dogs administered 20 or 100 mg/kg/day methoxychlor for 1 year, the levels of methoxychlor in the fat were 8.9 and 85 mg/kg, respectively (Hodge *et al.*, 1952). The levels of methoxychlor in the fat of rats exposed to 1.25, 10,

or 80 mg/kg/day for 2 years were 3.7, 2.3–6.8, and 11–22.7 mg/kg, respectively (Hodge *et al.*, 1952). In rats receiving 80 mg/kg/day, the highest levels of methoxychlor detected in the kidneys, liver, and brain were less than 4.2, 0.5, and 0.2 mg/kg, respectively (Hodge *et al.*, 1952).

Three days after oral administration of 3.6 or 11.6 mg/kg radiolabeled methoxychlor to lactating goats, radioactivity was detected in adrenals, brain, gall bladder, heart, kidneys, and liver (Davison *et al.*, 1982), thus indicating that absorbed methoxychlor and metabolites are widely distributed by the blood. However, the radioactivity in these tissues represented <1% of the administered dose. These observations are consistent with other information indicating that methoxychlor is rapidly metabolized and excreted from the body.

Methoxychlor and/or methoxychlor metabolites have been detected in milk following oral exposure of animals to methoxychlor during lactation. Accumulation in milk and/or elimination through lactation do not appear to be predominant dispositional fates for methoxychlor, but transfer of methoxychlor and methoxychlor metabolites to suckling rat pups via milk has been demonstrated. In lactating female goats given oral doses of 3.6 or 11.6 mg/kg radiolabeled methoxychlor, radioactivity in milk collected for 3 days was below limits of detection for one goat and represented only 0.065% of the dose given to the other goat (Davison *et al.*, 1982). In female rats fed 5, 50, or 150 mg/kg/day methoxychlor during late gestation and early lactation, milk levels of methoxychlor at postnatal day 7 were 25, 128, and 221% of plasma methoxychlor levels, respectively (Chapin *et al.*, 1997). Mono-hydroxy methoxychlor and bishydroxy methoxychlor, two major metabolites of methoxychlor, showed similar patterns of concentration in the milk with increasing methoxychlor exposure level. The data suggest that methoxychlor and metabolites concentrate in milk, relative to maternal plasma levels, after intermediate-duration dose levels of 50 mg/kg/day. Mean plasma levels of methoxychlor in suckling pups were <5 (below detection limit), 12.4, 37.8, and 59.9 ng/mL in the 0-, 5-, 50-, and 150-mg/kg/day groups, respectively (Chapin *et al.*, 1997). Mono-hydroxy methoxychlor was detected only in 150-mg/kg/day pup plasma (6.2 ng/mL), and bis-hydroxy methoxychlor was detected in the 50- and 150-mg/kg/day pup plasma (6.4 and 11.5 mg/kg/day). Pup plasma was not drawn for analysis until 27–30 hours after dams received the last dose; therefore, measured methoxychlor and metabolites may not have been indicative of peak body burden.

No studies were located that examined whether preconceptional or pregestational exposure of females to methoxychlor would result in exposure to the developing embryo/fetus or neonate. The evidence that methoxychlor is rapidly metabolized and eliminated from the body (e.g., all radioactivity from radiolabeled oral doses of 50 mg/kg methoxychlor was excreted by mice in feces and urine within 48 hours according to Kapoor et al. (1970)) suggests, however, that it is unlikely that methoxychlor at low background exposure levels would be stored in maternal tissues and subsequently mobilized during pregnancy or lactation. In an experiment (Swartz and Corkern, 1992), females from a litter produced after maternal exposure have ceased exhibited precocious puberty, but earlier offspring of these same dams exposed during gestation did not. It is unknown what mechanism produced these results.

A single study was located concerning the distribution of methoxychlor in animals after dermal exposure. Three days after two goats were administered a single dermal dose of 200 mg methoxychlor, low levels of methoxychlor (<0.3 mg/kg tissue) were detected in the skin, muscle, liver, fat, and kidneys (Davison *et al.*, 1983). Less than 0.1% of an applied dermal dose of methoxychlor was excreted in the milk of cows after 30 days (Ivey *et al.*, 1983; Skaare *et al.*, 1982). Interpretation of data from these studies is limited since only two goats were tested, and goats and cows may not be good models for dermal exposures in humans.

Metabolism

The metabolism of methoxychlor has been fairly well studied in vitro and in vivo in animals and with human liver microsomes (Lehman, 1952; Weikel and Laug, 1958). Both sets of data indicate that methoxychlor undergoes demethylation to form phenolic derivatives, with dechlorination and dehydrochlorination reactions occurring to a lesser extent.

Methoxychlor is metabolized mainly in the liver. The primary pathway by which methoxychlor is metabolized in the liver is sequential demethylation reactions to yield mono- and bis-hydroxy methoxychlor. Alternative names for these metabolites are 2-(p-methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane and 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane (sometimes abbreviated as HPTE). Dechlorinated metabolites such as bis-hydroxydiphenyl acetic acid and bis-hydroxybenzophenone have also been identified (e.g. Kapoor *et al.,* 1970). *In vitro* studies using human and rat liver microsomal preparations confirmed the generation of phenolic compounds via demethylation by hepatic cytochrome P450 (CYP) enzymes (Bulger and Kupfer, 1985; Bulger *et al.*, 1978a; Dehal and Kupfer, 1994; Elsby *et al.*, 2001; Kishimoto and Kurihara, 1996; Kupfer *et al.*, 1990; Kurihara and Oku, 1991; Stresser and Kupfer, 1997; Stresser and Kupfer, 1998; Stresser *et al.*, 1996).

The rapid demethylation of methoxychlor decreases its neurotoxicity and leads to a rapid elimination from the body (Lehman, 1952), making it significantly less toxic than other structural analogue, e.g. DDT. However, this detoxification pathway also is thought to act as an activation pathway for reproductive and developmental effects. Data from *in vitro* and *in vivo* rat studies indicate that the phenolic metabolites of methoxychlor resulting from demethylation (and contaminants in technical grade and laboratory grade methoxychlor) are responsible for most of the estrogenic activity rather than methoxychlor itself (Bulger *et al.*, 1978a; Bulger *et al.*, 1978b).

Elimination

No studies were located regarding excretion in animals after inhalation exposure to methoxychlor (ATSDR, 2002). Methoxychlor is excreted rapidly, predominantly in the feces and to a lesser extent in the urine (Kapoor *et al.*, 1970).
Three days after applying a single dose of 200 mg laboratory grade methoxychlor to the shaved backs of goats, 0.37–0.91% of the dose was excreted in the feces and 0.53–0.72% of the dose was excreted in the urine (Davison *et al.*, 1983).

4.1.2 Human information

No quantitative studies were located regarding neither absorption nor distribution, nor metabolism, nor elimination of methoxychlor from humans after exposure by any route. Moreover, no studies were located regarding excretion in humans after neither inhalation nor dermal exposure to methoxychlor (ATSDR, 2002).

4.1.3 Summary and discussion on toxicokinetics

No data were located concerning the toxicokinetics of methoxychlor in humans following any route of exposure, or in animals following inhalation exposure. Oral and cutaneous studies in animals indicate that methoxychlor is well absorbed by the gastrointestinal tract and to a lesser extent by the skin. However, some of the data from animal studies come from ruminant animals, which may have limited relevance to humans and other nonruminant species.

Once in the bloodstream, methoxychlor appears to distribute to most tissues of the body, with highest levels usually found in fat. Methoxychlor is metabolized 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.

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).

It should be noted that metabolism of methoxychlor decreases its neurotoxicity but leads to the formation of metabolites believed to enhance reproductive and developmental effects. In fact, data from *in vitro* and *in vivo* rat studies indicate that the phenolic metabolites of methoxychlor resulting from demethylation are responsible for most of the estrogenic activity rather than methoxychlor itself.

According to ATSDR (2002), the toxicokinetics of methoxychlor in humans is expected to be similar to the toxicokinetics of methoxychlor observed in animals.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

4.2.1.2 Acute toxicity: inhalation

4.2.1.3 Acute toxicity: dermal

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

A publication reports the development of fatigue and bruising several weeks after accidental exposure to a tomato pesticide dust containing methoxychlor and captan, followed by aplastic anemia after 2 months which proved fatal at 6 months (Ziem, 1982).

A single case study described neurological effects in a 21-year-old male acutely exposed (15-20 minutes) to a pesticide mixture that contained 15% methoxychlor and 7.5% malathion. The subject noted blurred vision and nausea 8-9 hours after exposure. He was admitted to the hospital 36 hours after exposure in a state of dehydration with severe abdominal cramps and diarrhea. Approximately 4 days later, he experienced dizziness and complete deafness followed by difficulty moving the extremities, hypoesthesias, parasthesias in the limbs, bilateral foot drop, and leg pain. There was no improvement in any of these neurological effects 6 years after exposure. The authors noted that neither methoxychlor nor malathion typically produced such profound effects, and attributed the special susceptibility of this individual to a deficiency in the enzyme responsible for the detoxification of malathion (ATSDR, 2002).

4.2.3 Summary and discussion of acute toxicity

Some case studies are available which report severe effect of methoxychlor after acute exposure to high doses.

4.3 Irritation

4.4 Corrosivity

4.5 Sensitisation

Methoxychlor has displayed little or no potential as a skin sensitizer (American Conference of Governmental Industrial Hygienists TLVs and BEIs, 2008).

4.6 Repeated dose toxicity

Observable changes in the liver (altered liver weight, altered enzyme and protein levels, pale and mottled appearance) and kidneys (cystic tubular nephropathy, elevated blood urea nitrogen [BUN]) of animals, 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).

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

4.6.1.2 Repeated dose toxicity: inhalation

4.6.1.3 Repeated dose toxicity: dermal

4.6.1.4 Repeated dose toxicity: other routes

4.6.2 Human information

Groups of volunteers were given methoxychlor at rates of 0, 0.5, 1, and 2 mg/kg/day for 8 weeks. Even the highest dosage was without detectable effect on health, clinical chemistry, or the morphology of blood, bone marrow, liver, small intestine, or testis (Krieger, 2001).

Pure erythroid aplasia was observed in a patient after an approximately 3-week chronic exposure to muchozol (an insecticide containing methoxychlor and pyrethrin). The patient recovered after 1 year (Sokołowski and Zawilska, 1983).

4.6.3 Summary and discussion of repeated dose toxicity

From a human health perspective, available data on the repeated dose toxicity of methoxychlor are limited to a study that found no clinical or histopathological changes in humans who ingested 2 mg/kg/d of methoxychlor for 6 weeks. These data are too limited to allow assessment of the hazard represented by methoxychlor for humans.

4.7 Mutagenicity

4.7.1 Non-human information

Methoxychlor was not mutagenic in bacteria, yeast or *Drosophila melanogaster*. Cytogenic and dominant lethal tests in mice were also negative (IARC, 1979).

4.7.1.1 In vitro data

4.7.1.2 In vivo data

4.7.2 Human data

Methoxychlor did not produce mutations at the thymidine kinase (TK) locus in human lymphoma cells, with or without metabolic activation (ATSDR, 2002).

4.7.3 Summary and discussion of mutagenicity

No evidence of mutagenicity could be drawn from the available data for experimental animals nor for humans.

4.8 Carcinogenicity

4.8.1 Non-human information

4.8.1.1 Carcinogenicity: oral

Methoxychlor was tested in one experiment in mice and in several experiments in rats by oral administration. The study in mice gave negative results. In at least four experiments in rats, dietary concentrations of 1000 mg/kg or more were used. A suggestion that it was hepatocarcinogenic, made in an earlier study that was inadequately reported, was not confirmed in three more recent experiments (IARC, 1979).

Subsequent to the meeting of the Working Group, the Secretariat became aware of a paper by Reuber (1979a), reporting the results of a study carried out in 1969 in which oral administration of methoxychlor induced testicular carcinomas in 27/51 male Balb/c mice, compared with 8/71 controls, but in none of the C3H mice tested. A further paper by Reuber (1979b) reported the results of a study carried out in 1951 in which oral administration of methoxychlor to Osborne-Mendel rats induced liver carcinomas (IARC, 1979).

The available data did not provide evidence that methoxychlor is carcinogenic in experimental animals (IARC, 1979).

4.8.1.2 Carcinogenicity: inhalation

4.8.1.3 Carcinogenicity: dermal

Methoxychlor was inadequately tested in mice by repeated skin application (IARC, 1979).

Other route of exposure

Methoxychlor was inadequately tested in mice by subcutaneous injection of single doses (IARC, 1979).

4.8.2 Human information

No case reports or epidemiological studies were available to the IARC Working Group when the evaluation was made in 1979 although IARC noted that the extensive production and the widespread use of methoxychlor over the past several decades, together with the persistent nature of the compound, indicated that widespread human exposure occurred. This was confirmed by many reports of its occurrence in the general environment and by its presence in human blood (IARC, 1979).

4.8.3 Summary and discussion of carcinogenicity

The available data did not provide evidence that methoxychlor is carcinogenic in experimental animals and no data were available to assess its potential for carcinogenicity in humans (IARC, 1979).

4.9 Toxicity for reproduction

4.9.1 Effects on fertility

4.9.1.1 Non-human information

4.9.1.2 Human information

4.9.2 Developmental toxicity

4.9.2.1 Non-human information

Methoxychlor is foetotoxic to animals (IARC, 1979).

A study was lead to demonstrate whether the observed differences in tissue growth between uterus and pituitary in response to methoxychlor administration were paralleled by a corresponding disparity in the expression of those growth factors (members of the vascular endothelial growth factor (VEGF) and angiopoietin families and their receptors) that are involved in the angiogenic cascade. Ovariectomized adult Sprague-Dawley female rats were administered methoxychlor (0-200 mg/kg, orally) for 1 or 3 weeks. Immunohistochemical staining of uteri and pituitaries was performed under strictly controlled conditions for VEGF and its receptor VEGFR2, Angiopoietin-1 (Ang1) and angiopoietin-2 and their tyrosine kinase receptor Tie2, and platelet endothelial adhesion factor (as an index of vascularity). Image acquisition and densitometric assessments of staining intensity were conducted under blind conditions. The results showed uterine methoxychlor -induced increases in the expression of VEGFR2 and Ang1, changes consistent with a normal proliferative response to oestrogenic stimulation. For VEGF, staining tended to be most pronounced in the stromal region, although there did not appear to be a progressive increase with dose. VEGFR2 expression showed significant dose-related trends in luminal and glandular epithelia by 1 week. Similar effects at 1 week were evident for Ang1 in glandular epithelium. In the anterior pituitary, a dose-related increase in VEGF was present for the 1 and 3 week treatments, and the number of pituitary vessels per unit area was also increased after 3 weeks. The effects indicate that even though the insecticide has not been found to cause an augmentation in pituitary growth, a dose-related rise in the expression of at least

one principal angiogenic factor is present that may be associated with an increase in vascular density (Goldman *et al.*, 2004).

Methoxychlor is an organochlorine pesticide that increases the rate of ovarian atresia. Because Bcl-2 (an antiapoptotic factor), Bax (a proapoptotic factor), gonadotropins, and estradiol are important regulators of atresia in the ovary, a study was lead firstly to examine whether methoxychlor -induced atresia occurred through alterations in Bcl-2 or Bax, and secondly to the examine the effect of methoxychlor on gonadotropins, estradiol, and their receptors. CD-1 mice were dosed with 8-64 mg/kg/d methoxychlor or vehicle (sesame oil). Ovaries were subjected to analysis of antral follicle numbers, Bcl-2, Bax, estrogen receptor, and follicle-stimulating hormone receptor levels. Blood was used to measure gonadotropins and estradiol. In some experiments, mice that overexpressed Bcl-2 or mice that were deficient in Bax were dosed with methoxychlor or vehicle and their ovaries were analyzed for atresia. Methoxychlor caused a dose-dependent increase in the percentage of atretic antral follicles compared with controls at the 32 and 64 mg/kg/d doses of methoxychlor. Methoxychlor treatment did not result in changes in Bcl-2 levels, but it did result in an increase in Bax levels in antral follicles. Methoxychlor treatment did not affect gonadotropin or estradiol levels, nor did it affect the levels of follicle-stimulating hormone or estrogen receptors. Mice that overexpressed Bcl-2 or mice that were deficient in Bax were protected from methoxychlor-induced atresia. These data suggest that methoxychlor induces atresia through direct effects on the Bax and Bcl-2 signaling pathways in the ovary (Borgeest *et al.*, 2004).

While *in vivo* studies indicate that methoxychlor exposure increases antral follicle atresia, in part by altering apoptotic regulators (Bcl-2 and Bax), they do not distinguish whether methoxychlor does so *via* direct or indirect mechanisms. An *in vitro* follicle culture system was used to test the hypothesis that methoxychlor is directly toxic to antral follicles, and that overexpression of anti-apoptotic Bcl-2, or deletion of pro-apoptotic Bax, protects antral follicles from methoxychlor -induced toxicity. Antral follicles were isolated from wild-type (WT), Bcl-2 overexpressing (Bcl-2 OE), or Bax deficient (BaxKO) mice, and exposed to dimethylsulfoxide (control) or methoxychlor (1-100 ug/mL) for 96h. Follicle diameters were measured every 24h to assess growth. After 96h, follicles were histologically evaluated for atresia or collected for quantitative PCR analysis of Bcl-2 and Bax mRNA levels. Methoxychlor (10-100 µg/mL) significantly inhibited antral follicle growth at 72 and 96h, and increased atresia (100 µg/mL) compared to controls at 96h. Furthermore, methoxychlor increased Bax mRNA levels between 48-96h and decreased Bcl-2 mRNA levels at 96h. While methoxychlor inhibited growth of WT antral follicles beginning at 72 h, it did not inhibit growth of Bcl-2 OE or BaxKO follicles until 96 h. Methoxychlor also increased atresia of small and large WT and BaxKO antral follicles over controls, but it did not increase atresia of large Bcl-2 OE antral follicles over controls. These data suggest that methoxychlor directly inhibits follicle growth partly by Bcl-2 and Bax pathways, and increases atresia partly through Bcl-2 pathways (Miller *et al.*, 2005).

Methoxychlor treatment of mice produced a mild uterotropic response as measured by increased uterine weight and epithelial height. Methoxychlor treatment of uterine Ishikawa cells *in vitro* induced Hoxa10 expression. Estrogen receptor (ER) binding to the Hoxa10 estrogen response element (ERE) was promoted by treatment with estradiol (E2); however, methoxychlor disrupted E2/ER/ERE complex formation and gel shift. Methoxychlor alone allowed weak ER/ERE binding. *In vivo* methoxychlor blocked the effect of E2 on Hoxa10 expression. Neonatal methoxychlor treatment resulted in an immediate suppression and cellular restriction of Hoxa10 expression as well as a permanent generalized decrease in expression that persisted in the adult. Methoxychlor inhibited the expression of Hoxa10, a gene necessary for uterine development and function. One common mechanism by which endocrine disrupting chemicals produce lasting reproductive tract defects is through permanent alteration of developmental gene expression (Fei *et al.*, 2005).

2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), the di-demethylated derivative of methoxychlor, inhibited the binding of (3)H-estradiol-17beta (3H-e2) to rat uterine cytosolic estrogen receptor *in vitro*, but methoxychlor did not. The possibility that the estrogenic activity of methoxychlor *in vivo* was due to biotransformation of methoxychlor was considered. The evolution of formaldehyde occurred when methoxychlor was incubated with rat hepatic microsomes in the presence of NADPH, indicating that methoxychlor was o-demethylated *in vitro*. The demethylation of methoxychlor was inhibited when methoxychlor was incubated with microsomes in the presence of hexobarbital or SKF-525a, suggesting the involvement of monooxygenase. The demethylated products were resolved by TLC into 3 distinct components. One of the products appears to be the di-demethylated derivative of methoxychlor, since it was identical to HPTE in 3 TLC systems (Bulger *et al.*, 1978a).

4.9.2.2 Human information

Human hepatoma cells (HepG2) were transiently transfected with either human or rat estrogen receptor alpha or estrogen receptor beta plus an estrogen-responsive, complement 3-luciferase construct containing a complement 3 gene promoter sequence linked to a luciferase reporter gene. After transfection, cells were treated with various concentrations of 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane in the presence (for detecting antagonism) or absence (for detecting agonism) of 17beta-estradiol. 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane was a potent estrogen receptor alpha agonist in human hepatoma cells cells, with EC_{50} values of approximately 5 10^{-8} and 10^{-8}mol/l for human and rat estrogen receptor alpha, respectively. In contrast, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane had minimal agonist activity with either human or rat estrogen receptor beta and almost completely abolished 17beta-estradiol-induced estrogen receptor beta-mediated activity. Moreover, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane behaved as an estrogen receptor alpha agonist and an estrogen receptor beta antagonist with other estrogen-responsive promoters (ERE-MMTV and vtERE) in human hepatoma cells and HeLa cells. This study demonstrates the complexity involved in determining the mechanism of action of endocrine-active chemicals that may act as agonists or antagonists through one or more hormone receptors (Gaido *et al.*, 1999).

4.9.3 Summary and discussion of reproductive toxicity

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. 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. In females, these interactions can result in disruption of estrus cyclicity, reduced fertility, and increased pre- and post-implantation losses. Effects in males can include delayed sexual maturation, atrophy of reproductive organs and accessory glands, and altered sexual or socio-sexual behavior. Many of these effects may be mediated through altered hormone levels (ATSDR, 2002).

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).

Data from oral studies in animals indicate that the reproductive system is the primary and most sensitive target of methoxychlor-induced toxicity in both adult and developing animals. Some metabolites and contaminants of methoxychlor are estrogenic or anti-androgenic and are capable of producing adverse effects on the male and female reproductive system. These effects are thought to

be mediated by interaction of methoxychlor or its metabolites with the estrogen receptor α, estrogen receptor β, or an as yet unknown estrogen receptor, or with the androgen receptor. These interactions can cause disruption of reproductive development or can alter reproductive function in adults. Altered serum and pituitary hormone levels have frequently been seen in animal studies, which may contribute to the changes in reproductive development and function. Developmental reproductive changes include precocious puberty and abnormal estrus cyclicity in females, delayed puberty in males, altered weights of reproductive organs and accessory glands, and impaired reproductive function in adulthood, including decreased pups/litter and increased resorptions. Similar effects have also been seen following exposure of adult animals. Additionally, gross and microscopic cellular changes have been observed in the reproductive organs of exposed adult females and males. While methoxychlor does have estrogenic properties, it is important to note that it is at least several thousand fold less potent than endogenous estrogen. There are no human data that report adverse effects on the reproductive system following exposure to methoxychlor, but in vitro studies reveal that human liver microsomes are capable of metabolizing methoxychlor to estrogenic compounds. Therefore, it is likely that methoxychlor could produce reproductive estrogen-like effects in humans (ATSDR, 2002).

In animals, signs of fetotoxicity (decreased fetal body weight, increased incidence of wavy ribs, resorptions, and death) were noted following exposure to methoxychlor *in utero*. These effects may be due to the maternal toxicity of methoxychlor and may not be true signs of teratogenicity (ATSDR, 2002).

4.10 Other effects

4.10.1 Non-human information

4.10.1.1 Neurotoxicity

Animal studies (oral exposure of animals) suggest that exposure to large amounts of methoxychlor can produce neurological effects, such as apprehension, nervousness, increased salivation, decreased locomotor activity, tremors, convulsions, and death. Methoxychlor has been demonstrated to be a neurotoxicant even in the absence of metabolism. 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).

4.10.1.2 Immunotoxicity

4.10.1.3 Specific investigations: other studies

4.10.2 Human information

4.10.3 Summary and discussion

Methoxychlor is a neurotoxic substance.

5 ENVIRONMENTAL HAZARD ASSESSMENT

EPIWIN v4.1 (US-EPA, 2011) predicts a PNEC QSAR of 0.012 mg/l and a lowest measured LC_{50} of 7.8 10^{-4} mg/l is reported in EPIWIN database, implying a high ecotoxicological potential.

5.1 Aquatic compartment (including sediment)

No ecotoxicological data is available for methoxychlor for algae and aquatic plants. However, some data are available from the literature for acute toxicity of methoxychlor on invertebrates (crustaceans and molluscs) and for long term toxicity of methoxychlor on fish. They are reported thereafter.

5.1.1 Toxicity data

5.1.1.1 Fish

5.1.1.1.1 *Short-term toxicity to fish*

5.1.1.1.2 *Long-term toxicity to fish*

Thorpe and its collaborators reported a valid chronic test for a 14-d exposure of *Oncorhynchus mykiss* to methoxychlor and a NOEC of 2.2 10^{-3} mg/l (Thorpe *et al.*, 2001).

Moreover, fish endocrine disruption should be further studied as a 15-day exposure to 100 µg/l methoxychlor caused significant proliferation of liver peroxisomes as indicated by increased peroxisomal surface and numerical densities and elevated activities of a peroxisomal oxidation enzyme (beta-oxidation enzyme acyl-CoA oxidase) as well as vitellogenin levels increased significantly in fish exposed in respect to controls. Moreover, a significant correlation between vitellogenin levels and the oxidase activity was found, suggesting early estrogenic effects that are associated with liver peroxisome proliferation (Ortiz-Zarragoitia and Cajaraville, 2005).

5.1.1.2 Aquatic invertebrates

5.1.1.2.1 *Short-term toxicity to aquatic invertebrates*

US Environment Protection Agency reports a valid acute test for a 96-h exposure of *Oronectes nais* to methoxychlor and a LC_{50} of 5 10^{-4} mg/l and another test consisting in 96-h exposure of *Crassostrea virginica* lead to an EC_{50} of 0.09 mg/l (US-EPA, 2009).

Other authors report a valid acute test for a 96-h exposure of *Asellus communis* to methoxychlor and a corresponding LC_{50} of 1.8 10^{-3} mg/l (Anderson and De Foe, 1980).

5.1.1.2.2 *Long-term toxicity to aquatic invertebrates*

Anderson and De Foe (1980) also reported a LC_{50} of 4.2 10^{-4} mg/l after a 28-d exposure of *Asellus communis* to methoxychlor. The NOEC for this test is not available.

5.1.1.3 Algae and aquatic plants

5.1.1.4 Sediment organisms

5.1.1.5 Other aquatic organisms

5.2 Terrestrial compartment

5.2.1 Toxicity data

5.2.1.1 Toxicity to soil macro organisms

5.2.1.2 Toxicity to terrestrial plants

5.2.1.3 Toxicity to soil micro-organisms

5.2.1.4 Toxicity to other terrestrial organisms

5.3 Atmospheric compartment

5.4 Microbiological activity in sewage treatment systems

5.4.1 Toxicity to aquatic micro-organisms

5.5 Non compartment specific effects relevant for the food chain (secondary poisoning)

5.5.1 Toxicity to birds

5.5.2 Toxicity to mammals

6 CONCLUSIONS ON THE PBT/VPVB ASSESSMENT

6.1.1 Assessment of PBT/vPvB properties – comparison with the criteria of Annex XIII

6.1.1.1 Persistence

While its photo-oxidation with hydroxyl radicals may be fast in certain conditions, main information available for methoxychlor allow to conclude on its persistency in the aquatic environment and in soils.

Therefore, methoxychlor shall be considered as a persistent (P) substance.

Key points to be discussed

Methoxychlor being degraded in anisoin, anisil, and 1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene (DMDE) (proportion depending on pH), these metabolites should be further considered also for their persistency properties leading standard tests (abiotic and biotic degradation). Furthermore, phenolic metabolites excreted by animals mainly via feces should be considered for their persistency as well given their potential for reproductive and developmental effects.

6.1.1.2 Bioaccumulation

QSAR models predicts a BFC of 1 044 and an experimental BCF of 70 700 is reported in EPIWIN database, implying a very high bioaccumulative potential. Even if some variations between species in the ability to metabolize this compound may exist, according to data available in the literature (BCF of 8 300), methoxychlor should be considered a very Bioaccumulative substance (vB).

Key points to be discussed

Methoxychlor is metabolized. Information about the bioaccumulation on metabolites is missing. Thus, methoxychlor metabolites should be further considered also for their bioaccumulative properties.

6.1.1.3 Toxicity

From a human health perspective, methoxychlor is rapidly and efficiently absorbed by the gatro-intestinal tract in experimental animals, distributed to tissues (preferently fatty), metabolized and its metabolites are being excreted. It should be noted that metabolism of methoxychlor decreases its neurotoxicity but leads to the formation of metabolites believed to enhance reproductive and developmental effects (e.g. estrogenic activity).

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. 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. In females, these interactions can result in disruption of estrus cyclicity, reduced fertility, and increased pre- and post-implantation losses. Effects in males can include delayed sexual maturation, atrophy of reproductive organs and accessory glands, and altered sexual or socio-sexual behavior. Many of these effects may be mediated through altered hormone levels.

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.

In animals, signs of fetotoxicity (decreased fetal body weight, increased incidence of wavy ribs, resorptions, and death) were noted following exposure to methoxychlor *in utero*. These effects may be due to the maternal toxicity of methoxychlor and may not be true signs of teratogenicity.

Methoxychlor is a neurotoxic substance. The mechanism by which methoxychlor, produces neurological effects could be linked to the membrane-association of a lipophilic species, which alters ion transport across neural membranes.

Available data on the repeated dose toxicity of methoxychlor to humans are limited to a study that found no clinical or histopathological changes in humans who ingested 2 mg/kg/d of methoxychlor for 6 weeks. These data are too limited to allow assessment of the hazard represented by methoxychlor for humans.

No evidence of mutagenicity could be drawn from the available data neither for experimental animals nor for humans.

The available data did not provide evidence that methoxychlor is carcinogenic in experimental animals and no data were available to assess its potential for carcinogenicity in humans.

From an environmental perspective, some ecotoxicological data are available for methoxychlor on invertebrates (short term data) and fish (long term data). Long-term data reported for fish are lower ($2.2\ 10^{-3}$ mg/l) than short term data reported for crustaceans (as low as $5\ 10^{-4}$ mg/l). Long term data for crustaceans are expected to be even lower than $5\ 10^{-4}$ mg/l, that is to say lower than the T cut-off criterion of 0.01 mg/l. The T criterion is believed to be fulfilled for methoxychlor from an environmental perspective but this should be consolidated by a long term data on invertebrates, preferably on crustaceans.

In summary, T criterion is fulfilled from an environmental perspective (EC_{50}< 0.01 mg/l and probable endocrine disruption) and from a human health perspective (substantial endocrine disrupting and reproductive effects).

Key points to be discussed

Methoxychlor is metabolized and available information about these metabolites toxicity show potential for *inter alia* endocrine disruption. Thus, methoxychlor metabolites should be considered in details also for their toxic properties.

6.1.2 Summary and overall conclusions on the PBT, vPvB properties

From an environmental perspective, methoxyclor should be considered a persistent (P) and very bioaccumulative (B) substance.

Considering the toxicological potential, T criterion is believed to be fulfilled for methoxychlor from an environmental perspective but this should be consolidated by a long term data on invertebrates, preferably on crustaceans. There is no evidence of mutagenicity and no data were available to assess its potential for carcinogenicity in humans but mammals and human health data show evidence of reprotoxicity even if methoxychlor is not classified for this property. Therefore, T criterion is fulfilled for methoxychlor as a human health perspective, but as methoxychlor is rapidly and efficiently absorbed and metabolised by organisms, it should be noted that these metabolites are believed to be responsible for most of the observed reprotoxic activity of the parent compound and thus, metabolites of methoxychlor should be investigated further for their P, B and T properties.

As a conclusion, methoxychlor should be considered a P (persistent), vB (very Bioaccumulative) and T (toxic) substance. Furthermore, its probable potential for long range transport makes it eligible as a POP substance.

INFORMATION ON USES AND EXPOSURE

1 MANUFACTURE AND USE(S)

1.1 Quantities

1.2 Identified uses

The major use is as active substance (insecticide) in pesticides (plant protection products and biocidal products).

Used as intermediate for chemical reactions

Process category

Environmental release category

Substance supplied to that use in form of

Market sector by type of chemical product

Sector of end use

Subsequent service life relevant for that use?

Used as intermediate for chemical reactions

Process category

Environmental release category

Substance supplied to that use in form of

Market sector by type of chemical product

Sector of end use

Subsequent service life relevant for that use?

2 POTENTIAL EXPOSURE AND RISK(S)

2.1 Workers

2.2 Consumers

2.3 Environment

2.4 Man exposed via the environment

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