Supporting information document to the draft proposal for listing Chlorpyrifos in Annex A to the Stockholm Convention on Persistent Organic Pollutants

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Physico-chemical properties

Table 1 Physico-chemical properties of chlorpyrifos and it's degradation and transformation products

	Chlorpyrifos	Chlorpyrifos-oxon (CPYO)	3,5,6-Trichloro-2-pyridinol (TCP)	2-Methoxy-3,5,6-trichloro-pyridine (TMP)
Property	Value and source	Value and source	Value and source	Value and source
CAS no.	2921-88-2	5598-15-2	6515-38-4	31557-34-3
Molecular weight [g/mol]	350.59	334.52	198.44	212.46
Form	tan, crystalline solid (94 % purity) (EC, 2005) Colourless to white crystalline solid (ILO & WHO, 2014)	No data	No data	No data
Odour	Mild mercaptan (experimental, 99.6 % purity) (EC, 2005)	No data	No data	No data
Melting point [°C]	41 – 42 (experimental at 97- 99 % purity) (EC, 2005) 42 at 99.9 % purity (Spain, 2017)	83.44 (estimated) (US-EPA, 2012)	82.30 (estimated) (US-EPA, 2012)	58.75 (estimated) (US-EPA, 2012)
Thermal decomposition point [°C] (decomposition before boiling)	170 – 180 Experimental data (EC, 2005; Spain, 2017)	No data	No data	No data

	Chlorpyrifos	Chlorpyrifos-oxon (CPYO)	3,5,6-Trichloro-2-pyridinol (TCP)	2-Methoxy-3,5,6-trichloro-pyridine (TMP)
Vapour pressure [Pa]	3.35 * 10^{-3} 25°C (purity 99.8%) (EC, 2005) 1.43 * 10^{-3} 20°C (purity 99.8%) (EC, 2005) 1.0 * 10^{-3} Experimental, 25°C (purity 98%) (WHO, 2009) 2.3 * 10^{-3} Compiled by Mackay et al. (2014)	8.87 * 10^{-4} (estimated) (US-EPA, 2012)	0.138 (estimated) (US-EPA, 2012) 3.57 *10^{-3} at 25°C 1.79 * 10^{-3} at 20°C (purity 99.6%) (Spain, 2017)	1.43 (estimated) (US-EPA, 2012) 1.27 at 25°C 0.9 at 20°C (purity 100%) (Spain, 2017)
Water solubility [mg/L]	1.05 at 20°C, in unbuffered solution, no pH dependency reported (EC, 2005) 0.39 at 19.5°C, pH not cited (98 % purity) (WHO, 2009) 0.73 Cited by Mackay et al. (2014) 0.941 (20°C, pH unknown, guideline EEC Method A6/OECD 105) Dow, as cited in WHO (2009) 0.588 (20°C, pH not stated, guideline OECD 105 flask method) Makhteshim, as cited in WHO (2009)	25.97 (25°C, estimated from log KOW) (US-EPA, 2012) 2623.4 (25°C, estimated from fragments) (US-EPA, 2012)	80.85 (25°C, estimated from log KOW) (US-EPA, 2012) 125.09 (25°C, estimated from fragments) (US-EPA, 2012)	60.36 (25°C, estimated from log KOW) (US-EPA, 2012) 750.88 (25°C, estimated from fragments) (US-EPA, 2012)

	Chlorpyrifos	Chlorpyrifos-oxon (CPYO)	3,5,6-Trichloro-2-pyridinol (TCP)	2-Methoxy-3,5,6-trichloro-pyridine (TMP)
Henry's Law constant [Pa m^3/mol]	1.09 (25°C) Cited by Mackay et al. (2014) 0.478, estimated (EC, 2005) 1.11 Cited by Mackay et al. (2014)	5.53 * 10^{-4} (25°C, QSAR estimated) (US EPA 2012) 1.142 * 10^{-2} (estimated from estimated vapour pressure and estimated water solubility) (US EPA 2012)	1.91 * 10^{-3} (25°C, QSAR estimated) (US EPA 2012) 3.370 * 10^{-1} (estimated from estimated vapour pressure and estimated water solubility) (US EPA 2012)	9.89 (25°C, QSAR estimated) (US EPA 2012) 5.021 (estimated from estimated vapour pressure and estimated water solubility) (US EPA 2012)
n-octanol/water partition coefficient (log KOW)	4.7 at 20°C, neutral pH, (EC 2005) 5.0 at 24.5°C (purity 98%), (WHO 2009) 4.96 - 5.11 at 20°C (Gebremariam et al., 2012) 5.2 - 5.267 at 25°C (Gebremariam et al., 2012)	2.89 (estimated) (US EPA 2012)	3.21 (experimental) (US EPA 2012)	No data
n-octanol/air partition coefficient (log KOA)	8.882 (estimated) (US EPA 2012) 8.34 Cited by Mackay et al. (2014)	9.541 (estimated) (US EPA 2012)	9.324 (estimated) (US EPA 2012)	5.669 (estimated) (US EPA 2012)
air/water partition coefficient (log KAW)	-3.922 Experimental database (US EPA 2012) -3.35 Cited by Mackay et al. (2014)	-6.651 (estimated) (US EPA 2012)	-6.114 (estimated) (US EPA 2012)	-2.399 (estimated) (US EPA 2012)

	Chlorpyrifos	Chlorpyrifos-oxon (CPYO)	3,5,6-Trichloro-2-pyridinol (TCP)	2-Methoxy-3,5,6-trichloro-pyridine (TMP)
Soil organic carbon/water partition coefficient (log KOC)	3.4 – 4.5 (mean: 3.9) (EC 2005) 3.7Experimental database (US EPA 2012) 3.93 cited by Mackay et al. (2014)	2.597 (estimated) (US EPA 2012) 2.618 (estimated) (US EPA 2012)	2.942 (estimated) (US EPA 2012) 3.188 (estimated) (US EPA 2012) 2.173 (PPDB 2020)	2.640 (estimated) (US EPA 2012) 3.111 (estimated) (US EPA 2012)

Persistence

Abiotic degradation

Table 2 Dependency on pH for abiotic degradation of chlorpyrifos

pH	Temperature	Half-lives	Reference
pH 5	25°C	73 d	McCall (1986)
pH 7	25°C	72 d	
pH 9	25°C	16 d	
pH 4.7	25°C	62.7 d	Meikle and Youngson (1978)
pH 6.9	25°C	35.3 d	
pH 8.1	25°C	23.1 d	

Soil Photolysis

1. In the study by Yackovich et al. (1985), the degradation of chlorpyrifos also did not differ significantly in light or dark. Since the study was not conducted according to current guidelines and a mercury lamp was used as the irradiation source, the study is only considered as additional information. Walia et al. (1988) irradiated chlorpyrifos under different photochemical conditions and showed that chlorpyrifos gives various photoproducts mainly by oxidative desulfuration, dehalogenation and hydrolytic processes under laboratory conditions. The study is also considered as additional information.

Rate of degradation in water

Table 3 Rate of degradation in water, laboratory studies

Water source	water	Half-life or DT_{50} (d)	Method of calculation	DT50 normalised to 12°C	Application (µg/L)	Temperature (°C)	pH	Salinity (%)	Oxygen content (%)	Total organic Carbon (mg/L)	Reference	remarks
Fröschweiher pond, Möhlin AG/Switzerland	pond	46 d	SFO	124.4 d	12.1	22.5°C	7.89	-/-	7.62	13.60	Gassen, 2015	High losses due to volatilisation, underestimation of DT50 values
Fröschweiher pond, Möhlin AG/Switzerland	pond	21 d	SFO	56.8 d	126	22.5°C	7.89	-/-	7.62	13.60		
Biederthal, France	Pond	2.78	FOMC	6.8 d	100.0	21.5 ± 0.2 °C.	8.08	-/-	9.70	10.64	Caviezel, 2015	High losses due to volatilisation, underestimation of DT50 values
	Pond, sterile	2.92	FOMC	7.2 d	100.0	21.5 ± 0.2 °C.	8.08	-/-	9.70	10.64		
	Pond	2.98	FOMC	7.3 d	10.0	21.5 ± 0.2 °C.	8.08	-/-	9.70	10.64		
Ynys Tachwedd, nr Borth, Ceredigion, Wales	Estuarine	45 d	SFO	59.8 d	40	15 °C	7.79	17	110	589.5	Swales, 2003	High losses due to volatilisation, underestimation of DT50 values
Borth Sands, Ceredigion, Wales	Coastal	35 d	SFO	35 d	40	12°C	7.83	36	114	812.9		
> 5 miles off shore from Plymouth, Devon, England	Open Sea water	75 d	SFO	51.3 d	40	8°C	8.06	38	112	645.3		
range		2.78 – 75 d		6.8 – 124.4								

Rate of degradation in soil

Table 4 Rate of degradation in soil, laboratory studies

Soil source	Soil texture	Half-life or DT_{50} (d)	Method of calculation	DT50 normalised to 12°C	Application (ppm)	Temperature (°C)	Soil moisture	pH	Organic Carbon (%)	Reference
Boone County, Missouri, USA	Silt Loam	21.43 9.55 (fast phase) 60.70 (slow phase)	DFOP	45.7 d 20.4 d (fast phase) 129.6 d (slow phase)	1.5 µg/g = 1000 g a.i./ha	20±2°C	50% MWHC	5.2/4.7[+]	1.6	Clark, 2013
Raymondville, Texas, USA	Sandy Clay Loam	5.964	SFO	12.7 d	1.5 µg/g = 1000 g a.i./ha	20 ± 2	50% MWHC	8.0/7.6	0.65	
MSL-PF, North Dakota, USA	Sandy Loam	9.6	FOMC	20.5 d	1.5 µg/g = 1000 g a.i./ha	20 ± 2	50% MWHC	6.4/6.2	1.7	
Tehama County, California, USA	Clay Loam	36.87 5.3 (fast phase) 49.19 (slow phase)	DFOP	78.7 d 11.3 d (fast phase) 105.0 d (slow phase	1.5 µg/g = 1000 g a.i./ha	20 ± 2	50% MWHC	6.7/6.4	1.3	
Marcham, UK	Sandy clay loam	22.25	FOMC	47.5 d	1.28 mg/kg = 960 g as	20±2°C	40% MWHC	7.7/8.3	1.7	De Vette and Schoonmade, 2001a
Charentilly, France	Silty clay loam	94.1	SFO	200.9 d	1.28 mg/kg = 960 g as	20±2°C	40% MWHC	6.1/8.0	1.0	
Cuckney, UK	Sand	110.3	SFO	235.4 d	1.28 mg/kg = 960 g as	20±2°C	40% MWHC	6.0/6.8	1.2	
Thessaloniki, Greece	Sandy silt loam	56.59	FOMC	120.8 d	1.28 mg/kg = 960 g as	20±2°C	40% MWHC	7.9/8.2	0.8	
Commerce, Miss.	Loam	11	Not reported	37.7 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	7.4	0.68	Bidlack, H.D., 1979
Barnes, N.D	Loam	22	Not reported	75.4 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	7.1	3.60	
Norfolk, VA	Loamy sand	102	Not reported	349.7 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	6.6	0.29	

Soil source	Soil texture	Half-life or DT_{50} (d)	Method of calculation	DT50 normalised to 12°C	Application (ppm)	Temperature (°C)	Soil moisture	pH	Organic Carbon (%)	Reference
Miami, IND	Silt loam	24	Not reported	82.3 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	6.6	1.12	
Catlin, ILL	Suilty clay loam	34	Not reported	116.6 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	6.1	2.01	
German 2.3, Germany	Sandy loam	141	Not reported	483.4 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	5.4	1.01	
Stockton, Calif	Clay	107	Not reported	366.9 d	6.7 ppm, 7.6 kg/ha	25ºC	75% 1/3 Bar	5.9	1.15	
Sultan, Washington USA	Silt loam	25 weeks = 175 d	Not reported	232.6 d	18 mg	**15°C**	20%	6.3	3.1	Getzin, 1981
		13 weeks = 91 d	Not reported	312.0 d		**25°C**				
		6 weeks = 42 d	Not reported	371.5 d		**35°C**				
Chehalis, Washington USA	Clay loam	4 weeks = 28 d	Not reported	96.0 d	18 mg	25°C	30%	5.7	7.0	
Semongok	clayey red yellow podzolic	77.0	1st order	264.0	5 µg/g	25°C	33%	4.8	2.2	Chai, 2013
		84.5	1st order	289.7	**25 µg/g**	25°C	33%	4.8	2.2	
Semongok, moisture dependence	clayey red yellow podzolic	120	1st order	411.4	5 µg/g	25°C	**air-dry soil**	4.8	2.2	
		77.0	1st order	264.0	5 µg/g	25°C	**field moisture content**	4.8	2.2	
		124	1st order	425.1	5 µg/g	25°C	**Wet (61 - 68%)**	4.8	2.2	
Semongok, temperature dependence	clayey red yellow podzolic	224	1st order	297.7	5 µg/g	**15°C**	33%	4.8	2.2	
		77.0	1st order	264.0	5 µg/g	**25°C**	33%	4.8	2.2	
		37.5	1st order	331.7	5 µg/g	**35°C**	33%	4.8	2.2	
Tarat	alluvial	53.3	1st order	182.7	5 µg/g	25°C	32%	5.6	1.8	
		76.2	1st order	261.3	**25 µg/g**	25°C	32%	5.6	1.8	
	alluvial	49.5	1st order	169.7	5 µg/g	25°C	**air-dry soil**	5.6	1.8	

Soil source	Soil texture	Half-life or DT_{50} (d)	Method of calculation	DT50 normalised to 12°C	Application (ppm)	Temperature (°C)	Soil moisture	pH	Organic Carbon (%)	Reference
Tarat, moisture dependence		53.3	1st order	182.7	5 µg/g	25°C	**field moisture content (32%)**	5.6	1.8	
		63	1st order	216.0	5 µg/g	25°C	**Wet (61 - 68%)**	5.6	1.8	
Tarat, temperature dependence	alluvial	83.5	1st order	111.0	5 µg/g	**15°C**	32%	5.6	1.8	
		53.3	1st order	182.7	5 µg/g	**25°C**	32%	5.6	1.8	
		36.5	1st order	322.9	5 µg/g	**35°C**	32%	5.6	1.8	
Balai Ringin	Red Yellow Podzolic soil	69.3	1st order	237.6	5 µg/g	25°C	22%	5.6	1.4	
		120	1st order	411.4	**25 µg/g**	25°C	22%	5.6	1.4	
Balai Ringin, moisture dependence	Red Yellow Podzolic soil	84.5	1st order	289.7	5 µg/g	25°C	**air-dry soil**	5.6	1.4	
		69.3	1st order	237.6	5 µg/g	25°C	**field moisture content (22%)**	5.6	1.4	
		63	1st order	216.0	5 µg/g	25°C	**Wet (61 - 68%)**	5.6	1.4	
Balai Ringin, temperature dependence	Red Yellow Podzolic soil	193	1st order	256.5	5 µg/g	**15°C**	22%	5.6	1.4	
		69.3	1st order	237.6	5 µg/g	**25°C**	22%	5.6	1.4	
		23.1	1st order	204.3	5 µg/g	**35°C**	22%	5.6	1.4	
range		6 - 224		12.7 - 483.4						

Rate of degradation in soil: termite control application rates

Table 5 Rate of degradation in soil, termite control application rates

Soil source	Soil texture	Half-life or DT_{50} (d)	Method of calculation	DT50 normalised to 12°C	Application (ppm)	Temperature (°C)	Soil moisture	pH	Organic Carbon (%)	Reference
										Racke 1993, Murray 2001
Seaford Rise, Australia	Red brown earth	462			1000 mg/kg	25°C	60% MWHC	7.1	1.2	Baskaran, 1999
Bedding material	Quarry sand	330			1000 mg/kg	25°C	60% MWHC	9.2	0.1	
Bedding material	Reidmix/sand-dolomite	315			1000 mg/kg	25°C	60% MWHC	9.6	0.2	

Rate of degradation in soil: field studies

Table 6 Rate of degradation in soil, field studies

Soil source	Soil texture	Half-life or $DisT_{50}$ (d)	Method of calculation	Application (kg a.i/ha)	Depth	Soil moisture	pH	Organic Carbon (%)	Reference
Geneseo (Illinois), cropped soil	Silt loam	88.89	SFO	3.36 kg a.i/ha	0-15 cm	45.89 %w/w	5.9	1.6	Fontaine, D.D et al. (1987)
Midland (Michigan), cropped soil	Sandy Loam	30.04	SFO	3.36 kg a.i/ha	0-15 cm	23.10 %w/w	7.7	1.3	
Davis (California), cropped soil	Loam	29.18	SFO	3.36 kg a.i/ha	0-15 cm	42.04 %w/w	7.9	0.75	
Tranent, Scotland, bare soil	Sandy clay loam	7.86 d	SFO	0.960 kg a.i./ha	0-10, 10-20 cm	Not reported	6.7	1.9	Old, J. (2002a)
Charentilly/ Tours, France, bare soil	Clay loam	11 d		0.960 kg a.i./ha	0-10, 10-20 cm	Not reported	7.1	1.1	Old, J. (2002b)
Valtohori/ Thessaloniki, Greece, bare soil	Sandy silt loam	9.022 2.24 (fast) 61.67 (slow)	DFOP	0.960 kg a.i./ha	0-10, 10-20 cm	Not reported	8.0	0.9	Old, J. (2002c)
Tivenys/ Tarragona, Spain, bare soil	Clay loam	0.323 0.09 (fast) 5.42 (slow)	DFOP	0.960 kg a.i./ha	0-10, 10-20 cm	Not reported	8.2	1.4	Old, J. (2002d)
Range		5 – 89 d							

Rate of degradation in water-sediment studies

Table 7 Rate of degradation in water-sediment studies, laboratory studies

Sediment source	Sediment texture	Half-life or $DegT_{50}$ total system (d)	DT50 total system normalised to 12°C	DisT50 water (d)	DisT50 sediment (d)	Application (ppm)	Temperature (water) (°C)	pH sediment	pH water	Organic Carbon (%) sediment	Organic Carbon (%) water	Reference
Brown Carrick Sediment	Sandy loam	22 d	-/-	3 d		960 g a.i./ha	Not given	5.2	7.4	2.5	0.0016	Reeves, G.L. and Mackie, J.A., 1993
Auchingilsie Sediment	Clay loam	51 d	-/-	6 d		960 g a.i./ha	Not given	6.3	6.7	3.2	0.00172	
Pond sediment	Silty Clay Loam	30.5	104.6	Not given	Not given		25°C	7.7	8.1	3.1	Not given	Kennard, 1996
Calwich Abbey Lake, Staffordshire, UK	Silt loam	30.67 (SFO)	65.5	3.075 (SFO)	3.007 (HS)	0.54 mg a.i../L	20 ± 2°C	7.5	7.71	5.8	Not given	Kang, 2015, kinetics calculated by Abu, A., 2015d
Swiss Lake, Chatsworth, Derbyshire, UK	Sand	58.25 (SFO)	124.3	5.063 (SFO)	34.49 (SFO)	0.54 mg a.i../L	20 ± 2°C	7.0	7.84	0.7	Not given	
range		22 – 58.25 d	65.5 – 124.3 d									

Other evidence of persistence

According to a 10-year water quality assessment study performed by the United States Geological Survey, chlorpyrifos was the most heavily used and frequently detected insecticide; it was found at concentrations exceeding an aquatic-life benchmark of 0.04 m g/L for water in 37% samples collected from water bodies with diverse land-use settings throughout the USA (Gilliom et al., 2006). Chlorpyrifos was detected frequently in both urban and rural streams and major rivers in the USA, but less frequently in groundwater samples (Kolpin et al., 2000).

Bioaccumulation

Table 8 Bioaccumulation studies assessed for evaluation of chlorpyrifos

publication	species	endpoint type	endpoint value	unit	comments
plants					
Prasertsup and Ariyakanon (2011)	duckweed (*Lemna minor*)	BCF	5700	mL/g	BCF calculated on daily measurements
Prasertsup and Ariyakanon (2011)	water lettuce (*Pistia stratiotes*)	BCF	3000	mL/g	BCF calculated on daily measurements
Lal et al. (1987)	Blue-Green Algae *Anabaena* sp.	BCF	678	mL/g	concentration of test substance not maintained, no calculations reported
Lal et al. (1987)	*Aulosira fertilissima*	BCF	397	mL/g	concentration of test substance not maintained, no calculations reported
macroinvertebrates					
Serrano et al. (1997)	*Mytilus galloprovincialis*	BCF	400 ± 119	mL/g	concentration of test substance within 25% fluctuation
Thacker et al. (1992)	eastern oyster (*Crassostrea virginica*)	BCF	950 (whole oysters); 1600 (tissue fraction)	mL/g	significant dip in CPY by day 21 (56%); chlorpyrifos concentration low in shell liquor
Woodburn et al. (2003)	eastern oyster (*Crassostrea virginica*)	BCF	565 (whole oyster); 1400 (oyster tissue)	mL/g	chlorpyrifos concentration low in shell liquor
Rubach et al. (2010)	15 macroinvertebrate species	BCF	100 - 13930	mL/g	C14 labelling of chlorpyrifos at the di-ethyl-phosphorothiol branch
Montañés et al. (1995)	*Asellus aquaticus*	BCF	1715	mL/g	Mesocosm experiment with time dependant significantly reduced survival
amphibia					

publication	species	endpoint type	endpoint value	unit	comments
Robles-Mendoza et al. (2011)	axolotl (*Ambystoma mexicanum*)	BCF	3632	mL/g	decrease in chlorpyrifos concentration by 50% during exposure; behavioural effects
A. Jantunen et al. (2008)	*Lumbriculus variegatus*	BSAF	Range of 6 to 99		steady state not reached
Fish					
Hansen et al. (1986)	gulf toadfish (*Opsanus beta*)	BCF	5100	mL/g	toxic effects; increased mortality at 150 µg/L for which the BCF of >5000 was reported
Welling and Vries (1992)	guppies (*Poecilia reticula*)	BCF	1847	mL/g	fish not fed during two week experiment; chlorpyrifos concentration decreased by 90%
Mulla et al. (1973)	channel catfish (*Ictalurus punctatus*)	BCF	4677	mL/g	extreme fluctuations in temperature and O2 concentratrion; fish analysed without gut
Mulla et al. (1973)	black crappie (*Pomoxis nigromaculatus*)	BCF	3333	mL/g	extreme fluctuations in temperature and O2 concentratrion; fish analysed without gut
Mulla et al. (1973)	largemouth bass (*Micropterus salmoides*)	BCF	1333	mL/g	extreme fluctuations in temperature and O2 concentratrion; fish analysed without gut
Mulla et al. (1973)	bluegill (*Lepomis microchirus*)	BCF	1200	mL/g	extreme fluctuations in temperature and O2 concentratrion; fish analysed without gut
Jarvinen et al. (1983)	fathead minnow (*Pimephales promelas*)	BCF	1673 ± 423	mL/g	toxic effects
Deneer (1993)	guppy (*Poecilia reticulata*)	BCF	1580	mL/g	BCF calculated in Gisey et al. 2014

publication	species	endpoint type	endpoint value	unit	comments
Thomas and Mansingh (2002)	red hybrid tilapia (*Oreochromis* sp.)	BCF	116 (semi static exposure); 3313 (pulse exposure)	mL/g	high fluctuation of chlorpyrifos; steady state not reached; Dursban 25 C used
J. Eaton et al. (1985)	bluegills (*Lepomis microchirus*)	BCF	600	mL/g	toxic effects; high fluctuation in chlorpyrifos concentration; Lorsban 4C used
J. Eaton et al. (1985)	fathead minnow (*Pimephales promelas*)	BCF	1150	mL/g	toxic effects; high fluctuation in chlorpyrifos concentration; Lorsban 4C used
Goodman, Hansen, Cripe, et al. (1985)	california grunion (*Leuresthes tenuis*)	BCF	1000	mL/g	significant mortality and toxic effects; control fish contaminated with chlorpyrifos
Cripe et al. (1986)	sheepshead minnows (*Cyprinodon variegatus*)	BCF	1830	mL/g	mortality in high concentrations; different feeding regiments tested; BCF incresed with CPY concentration and higher feeding rates
Goodman, Hansen, Middaugh, et al. (1985)	*Menidia beryllina*	BCF	440	mL/g	steady state not reached; mortality in higher concentrations
Goodman, Hansen, Middaugh, et al. (1985)	*Menidia peninsulae*	BCF	580	mL/g	steady state not reached; mortality in higher concentrations; negative effect of solvent
Macek et al. (1972)	bluegills (*Lepomis microchirus*)	BCF	2304	mL/g	extreme fluctuations in temperature and O2 concentratrion; behavioural effects
Macek et al. (1972)	largemouth bass (*Micropterus salmoides*)	BCF	1440	mL/g	extreme fluctuations in temperature and O2 concentratrion; behavioural effects
Deneer (1994)	three-spined stickleback (*Gasterosteus aculeatus*)	BCF	1057	mL/g	insufficient information on BCF calculation
Tsuda et al. (1992)	carp (*Cyprinus carpio*)	BCF	410 ± 100	mL/g	steady state not reached

publication	species	endpoint type	endpoint value	unit	comments
Tsuda et al. (1997)	guppies (*Poecilia reticulata*)	BCF	1506 (female guppy), 2305 (male guppy)	mL/g	steady state not reached
Tsuda et al. (1997)	medaka (*Oryzias latipes)*	BCF	1561	mL/g	steady state not reached
Tsuda et al. (1997)	goldfish (*Carassius auratus*)	BCF	763	mL/g	steady state not reached
Tsuda et al. (1997)	white cloud mountain minnow (*Tanichthys albonubes)*	BCF	745	mL/g	steady state not reached
report no ES-928 (J42) in Spain (2017)	rainbow trout (*Onchorhynchus mykiss*)	BCF	1374 ± 321	mL/g	not normalized for lipid or growth
El-Amrani et al. (2012)	zebrafish (*Danio rerio*)	BCF	5011	mL/g	not normalized for lipid; eleuthero embryos with 11 - 20% lipid content
Alharbi et al. (2017)	medaka (*Oryzias latipes)*	BCF	2691	mL/g	not normalized for lipid; eleuthero embryos with 11 - 20% lipid content
monitoring data					
Landers et al. (2008)	white fir (*Abies concolor)*	chlorpyrifos concentration	first year not detected, second year 19.7	ng/g lipid weight	
Landers et al. (2008)	lodgepole pine (*Pinus contorta*)	chlorpyrifos concentration	first year 11.6 , second year 20.5	ng/g lipid weight	
Aston and Seiber (1997)	*Pinus ponderosa*	BCF_m	9800	mass: mass ratio	combined from wax cuticle and cell
Kurt-Karakus et al. (2011)	zooplankton	BAF	up to 117000		possible adsorption
Jessup et al. (2010)	sea otters (*Enhydra lutris* ssp.)	concentration in blood serum	maximum 342.6	ng/g lipid weight	
Stansley et al. (2010)	river otters (*Lontra canadensis*)	concentration in liver tissue	maximum 6.91	ng/g wet weight	

publication	species	endpoint type	endpoint value	unit	comments
Adrogué et al. (2019)	blackbrowed albatross (*Thalassarche melanophris*)	concentration in feathers	58.64 ± 27.31 (male); 49.56 ± 18.45 (female)	ng/g	feathers washed with deionized water before analysis
Adrogué et al. (2019)	cape petrels (Daption capense)	concentration in feathers	84.88 ± 50.57 (male); 75.98 ± 47.97 (female)	ng/g	feathers washed with deionized water before analysis
Morris et al. (2014)	mushrooms, lichen and green plants	BCFv	8.0 - 8.7	mass: mass ratio	recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2014)	caribou:vegetation	BMF	1.6 ± 0.31 (spring); 1.4 ± 0.43 (summer; 2.1 ± 0.64 (fall/winter)		recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2014)	wolf:caribou	BMF	0.078 ± 0.019		recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2014)	$wolf_{liver}$:$caribou_{liver}$	BMF	1.7 ± 0.52		recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2014)	green plants	TMF	0.61 (0.47 - 0.79)	pg/g lipid weight	recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2016)	plankton	BAF	7 943 282 ± 5 011 872	mL/g	recovery rate of chlorpyrifos from biota samples 52 ±17%
Morris et al. (2016)	polar bear fat: seal blubber	BMF	1.3 ± 0.22 and 0.90 ± 0.27		recovery rate of chlorpyrifos from biota samples 52 ±17%; concentration in seal blubber not reported; detection in seal blubber below 20%
Morris et al. (2016)	seal blubber	TMF	0.27, 0.57 and 0.18		recovery rate of chlorpyrifos from biota samples 52 ±17%; concentration in seal blubber not reported; detection in seal blubber below 20%

publication	species	endpoint type	endpoint value	unit	comments
Singh et al. (2008)	chicken	mean concentration in blood	80	ppb	
Singh et al. (2008)	goat	mean concentration in blood	70	ppb	
Singh et al. (2008)	man	mean concentration in blood	40	ppb	
Shaker and Elsharkawy (2015)	buffalo	concentration in raw milk	1.870 – 3.514	mg/kg	
Weldon et al. (2011)	*Homo sapiens*	concentration in breast milk	urban mean 40.5; agricultural mean 139	pg/g milk	
Bedi et al. (2013)	*Homo sapiens*	concentration in breast milk	median 1664.2	ng/g lipid weight	
Sanghi et al. (2003)	*Homo sapiens*	concentration in breast milk	mean value 0.230 ± 0.024	mg/kg	

Table 9 Bioaccumulation studies not used for assessment but used in EC 2017b

in summary as report number	species	endpoint type	endpoint value	unit	Publicly available
GHE-T-281 (J061)	Eel (*Anguilla anguilla*)	BCF	400	mL/g	no
GS 1318 (J41)	mosquito fish (Gambusia sp.)	BCF	65 - 472	mL/g	no
DECO-ES-2377 (J66)	Eastern oyster (*Crassostrea virginica*)	BCF	430	mL/g	no

2. Details of bioaccumulation studies not listed in the dossier:

3. Asselus aquaticus was exposed to chorpyrifos in the form of Drusban 4E. The nominal concentration of active substance were 0.7 and 5 µg/L (Montañés et al., 1995). Exposure took place in nature-like mesocosms, 40 m long ditches lined with water-tight, non-toxic PVC and a 0.25 m sediment layer and filled with water drawn from a underground well. Polythene spheres were used to hold 10 animal each. 120 animals per concentration were exposed this way. 50 animals were used as controls in a ditch without chlorpyrifos. Water samples were taken at 15 min and at 1, 2, 4, 7, 14 and 29 days after application. On days 1, 2, 3, 4, 6, 8, 13, 17 and 23 animals were sampled by harvesting one or two spheres. The recovery rate from biota was 54 + 4% and 82 + 5% from water. The limits of detection were 0.001 µg/L in water and 200 ng/g lipid weight (lw) for Asselus aquaticus. The concentration of chlorpyrifos was not stable and declined continuously over the course of the experiment with a decline above 25% in the first three days. Survival was significantly reduced in the course of the experiment, the authors noted that this may be due to predation or toxicity of chlorpyrifos. An average lipid content of 0.69 ± 0.26% was observed. Kinetic BCF were calculated for days two to seventeen. On average the BCF was 1715.

4. A BCF of 1700 for juvenile guppies (*Poecilia reticula*) was reported in a 14-day static exposure with chlorpyrifos (Welling & Vries, 1992). This study is considered unsuitable for BCF calculation as the nominal concentration of 10 µg/L decreased to below 1 µg/L by day 9. Furthermore, fish were not fed during the experiment.

5. Jarvinen et al. (1983) exposed fathead minnows (*Pimephales promelas*) to chlorpyrifos in a 200-day full life cycle experiment under flow through conditions. A BCF of 1673 ± 423 for first generation minnows at 60 days was calculated. Steady state was assumed. Effects occurred proportional to acetylcholinesterase inhibition. At the highest concentration of 2.68 µg/L reduction of growth, deformities and later significant mortality occurred. Growth reduction was also observed for 1.21 µg/L, later in the test. Sexual maturation and reproduction were reduced in all exposure groups at concentrations as low as 0.12 µg/L. In the second generation, deformities occurred more frequently and at lower water concentrations. Based on the toxic effects, the BCF should be interpreted with caution.

6. Deneer (1993) calculated uptake and elimination constants for the guppy (*Poecilia reticulata*) under flow through conditions of 2 µg/L chlorpyrifos. The experiment lasted 24 days, 20 days of exposure and four days for depuration. The uptake constant was calculated as 7000 ± 2000 L/kg/d, the depuration constant as 0.40 ± 0.11 L/kg/d. The BCF was calculated in (Giesy et al. 2014) as 1580. Steady state was not reached and chlorpyrifos concentration showed a high fluctuation.

7. Thomas and Mansingh (2002) conducted two experiments exposing red hybrid tilapia (*Oreochromis* sp.) to the commercial product Dursban 25 C with 25% chlorpyrifos active ingredient. A three-day semi-static exposure, with the water concentration fluctuating between 48 µg/L and 35 µg/L chlorpyrifos, resulted in a BCF of 116. A four-day pulse exposure with water concentration between 4.9 µg/L and 3.6 µg/L resulted in a BCF of 3313. This study must be interpreted with caution, as steady state was not reached and the concentration of chlorpyrifos fluctuated highly. Moreover, Dursban 25 C contains other ingredients that can have effects on fish.

8. Artificial streams were exposed to Lorsban 4E with 40,7% active ingredient chlorpyrifos in a 100-day experiment (J. Eaton et al., 1985). One stream was continuously dosed, the other was subjected to pulse exposure every two weeks. Water concentration in the continuously dosed stream varied

between 0.12 µg/L and 0.83 µg/L during the 100 days and also spatially between the sections of the stream up to 0.17 µg/L. The pulsed stream reached maximum concentrations of up to 7 µg/L directly after pulse events. Both streams received the equivalent amount of chlorpyrifos during the experiment. Bluegills *(Lepomis microchirus)* and fathead minnows (*Pimephales promelas*) were exposed. For fathead minnows deformities occurred in the pulse experiment only, reproductive losses and decreased body weight of second generation fish occurred in both streams. For bluegills behavioural effects occurred. Both fathead minnows and bluegills showed acetylcholinesterase inhibition. For fatheaded minnow a tissue BCF of 760 was calculated and a lipid BCF of 23000. Normalised to 5% lipid content the BCF is 1150. For bluegill a tissue BCF of 100 was calculated and a lipid BCF of 12000, which gives a BCF of 600 when normalised to 5% lipid content. These values should be interpreted with caution as Lorsban was used instead of pure chlorpyrifos, in addition chlorpyrifos concentrations were not constant and toxic effects occurred. Additionally, the authors did not specify which tissue was analysed nor give the lipid content of the fish.

9. In a 30-day early life stage toxicity test, the california grunion (*Leuresthes tenuis*) was exposed to 0.14 µg/L chlorpyrifos under flow through conditions (Goodman, Hansen, Cripe, et al., 1985). A BCF of 1000 was determined. This result should be interpreted with caution, as chlorpyrifos residue was also found in fish sampled from the seawater and solvent control.

10. A BCF of 1830 was determined for sheepshead minnows (*Cyprinodon variegatus*) in a 28-day early life stage toxicity test under flow through conditions (Cripe et al., 1986). The effect of different feeding ratios and chlorpyrifos concentrations were examined. Fish were exposed to 10 different concentrations ranging from to 0.6 µg/L to 52 µg/L and three different feeding regiments. BCF increased with increasing chlorpyrifos concentrations and increasing amount of feed. These results should be interpreted with caution as significant mortality occurred in higher concentrations.

11. Different silverside species were exposed to chlorpyrifos in a 28-day early life stage toxicity test under flow through conditions (Goodman, Hansen, Middaugh, et al., 1985). For *Menidia beryllina* a BCF of 440 was determined. For *Menidia peninsulae* a BCF of 580 was reported. BCFs increased with higher chlorpyrifos concentrations. Results should be interpreted with caution as mortality occurred in higher concentrations for both fish species and *M. peninsulae* survival was negatively affected by the solvent used.

12. Macek et al. (1972) described the uptake of chlorpyrifos in bluegills *(Lepomis microchirus)* and largemouth bass (*Micropterus salmoides*) during a 63-day field study with chlorpyrifos applied at mosquito larvicide rates to small ponds. Two applications were performed on day one and day 35. The maximum BCF for bluegill was 2304 on day seven and 1440 BCF for largemouth bass on day three. Water temperatures could rise up to 31 °C as the experiment was conducted during summer months. This influenced the solved oxygen, which could drop below 50%. Behavioural effects were noted shortly after each application. Results should be interpreted with caution as the variation of chlorpyrifos concentrations exceeded the 20% window.

13. For the three-spined stickleback (*Gasterosteus aculeatus*) a BCF of 1057 was derived from a 30-day laboratory experiment (Deneer, 1994). Fish were exposed to chlorpyrifos at 0.19 ± 0.03 µg/L for 21 days under flow through conditions, depuration lasted 9 days. Insufficient information is reported on BCF calculation, therefore the BCF value should be interpreted with caution.

14. Tsuda et al. (1992) exposed carp (*Cyprinus carpio*) to 0.49 ± 0.11 µg/L chlorpyrifos during a 14-day flow through experiment. A BCF of 410 ± 100 was calculated on day 14. Although steady state was not reached, the BCF was calculated as it would be under steady state conditions. The same reason

for caution applies to the study from Tsuda et al. (1997) with the same experimental set up, where a BCF of 2406 was calculated for male guppies (*Poecilia reticulata*), a BCF of 1464 calculated for female guppies, 1561 for medaka (*Oryzias latipes)*, 763 for goldfish (*Carassius auratus*) and 745 for white cloud mountain minnow (*Tanichthys albonubes)*.

15. Chlorpyrifos and its transformation product chlorpyrifos oxon were detected in needles of potted ponderosa pines at three sites in California in 1994 (Aston & Seiber, 1997). Needle compartments were analysed separately and included a wash for polar and non polar adsorbed substances, the waxy cuticle and the remainder needle. Values for chlorpyrifos residue in each compartment were combined to calculate total burden per sample. Two sites were sampled, one was located at the edge of the Central Valley (114 m altitude), while the others were situated at higher altitudes in the Sequoia National Park (533 and 1920 m, resp.). The detection frequency was significantly higher at the site in the Central Valley than those at the other two locations. The maximum level of chlorpyrifos in pine needles, which was found at the site in the Central Valley, amounted to ca. 129 ng/g dry weight, while the maximum level of chlorpyrifos oxon was about 110 ng/g dry weight at the same location[1]. Assuming that the needles of the potted pines, located at the site in the Central Valley, were in equilibrium with the compound in the surrounding air after 10 weeks of exposure, the vegetation: air BCF_m[2] was estimated as 9800.

16. Shaker and Elsharkawy (2015) detected chlorpyrifos in raw buffalo milk samples offered for sale in the Egyptian city of Assiut in 2013. The compound was found in 33 % of the samples. The average concentration was 3.01 ± 1.0 mg/kg. All measured values significantly exceeded the maximum residue level of 0.01 mg/kg set by the European Commission (EC, 2008) for chlorpyrifos. Contaminated feed, grass or corn silage, and direct application on dairy cattle were assumed as the main sources of the chlorpyrifos residues in milk.

Long range Transport Potential – Additional Information

publication	medium	time frame	concentration	handling of blanks
Chernyak et al. (1996)	fog condensate sea water melting ice	1993	5 ng/L max. 65 pg/L max. 170 pg/L	blanks analysed as field samples, no chlorpyrifos detected
Garbarino et al. (2002)	snow	1995/96	70 – 80 ng/L	no information
Hermanson et al. (2005)	ice core	1972 – 1990	max. 16.2 ng/L	concentrations blank corrected
Ruggirello et al. (2010)	ice core	1971 – 2005	max. 808 pg/cm²/year	Method detection limit (MDL, defined as mean blank value + 3 x SD of blank values)

[1] The concentration values were estimated from a diagram of the cited publication.

[2] In this study the BCF_m was defined as the mass : mass ratio of the concentration of a chemical in vegetation tissues to its concentration in air.

publication	medium	time frame	concentration	handling of blanks
Muir et al. (2004)	lake water	1998 – 2001	mean 0.27 ng/L	MDL
Landers et al. (2008)	snow	2003	0.010 - 0.030 ng/L	On average concentrations found in blanks were 3% of the concentration in snowpacks and the concentration in blanks was subtracted from concentrations found in snow samples.
L. M. Jantunen et al. (2007)	air	2007	0.36 to 30.4 pg/m³	no information
Pućko et al. (2015)	air	2008	3.1 ± 1.9 pg/m³	no information
Pućko et al. (2015)	sea water	2008	31 ± 19 pg/L	no information
Hung et al 2013 Hung et al. in Balmer et al. (2019)	air	2006 - 2009	<MDL – 6.8 pg/m³	MDL
Zhong et al. (2012)	air	2010	1 - 146 pg/m³	MDL
Zhong et al. (2012)	sea water	2010	0.1 - 111 pg/L	MDL
Pućko et al. (2017)	snow	2012	mean ± SD, 4.8 ± 1.3 pg/L	MDL
Pućko et al. (2017)	melt pond water	2012	mean ± SD, 14.4 ± 2.5 pg/L	MDL
Pućko et al. (2017)	sea water	2012	mean ± SD, 14.1 ± 6.0 pg/L (0m), 10.5 ± 1.7 pg/L (5m)	MDL
Pućko et al. (2017)	air	2012	mean ± SD, 0.10 ± 0.04 pg/m³	MDL
L. M. Jantunen et al. (2015)	air	2007, 2008, 2010, 2011 and 2013	mean ± SD, 1.1 ± 1.3 pg/m³	No chlorpyrifos measured in blanks, instrumental detection limits 0.02 pg/m³ and 0.1 pg/L
L. M. Jantunen et al. (2015)	water	2007, 2008, 2010, 2011 and 2013	mean ± SD, 13 ± 12 pg/L	No chlorpyrifos measured in blanks, instrumental detection limits 0.02 pg/m³ and 0.1 pg/L
Bigot et al. (2017)	sea ice	2015	5.2 – 12.0 pg/L	MDL

publication	medium	time frame	concentration	handling of blanks
Bigot et al. (2017)	sea water	2015	0.74 – 1.0 pg/L	MDL
Bigot et al. (2017)	snow	2015	6.2 – 11.5 pg/L	MDL
Boström (2020)	air	2009 - 2018	median concentrations of 0.002 ng/m^3	no information
Boström (2020)	precipitation	2002 – 2018	max. concentrations between 0.0001 and 0.01015 µg/L	no information

17. Muir et al. (2004) compared their findings of current-use pesticides in remote areas with the predicted atmospheric half-lives and characteristic travel distances (CTDs). Predicted half-lives in air of the most current-use pesticides do not exceed the Stockholm criterion for LRTP. The authors discussed that the discrepancy between modelling data and monitoring findings is due to an overestimation of the atmospheric OH radical concentration applied in the model calculations. Furthermore, precipitation scavenging may be overestimated by LRTP models assuming a high ability of current-use pesticides to dissolve in rain droplets. If the atmosphere is sufficiently cold, cloud water and falling hydrometeors will be frozen and have a much smaller capacity to take up water-soluble organic chemicals. Snow may have a considerably lower scavenging efficiency for the vapours of water-soluble pesticides compared to that of rain. Snow may limit the LRTP of these pesticides much less than rain. The accuracy of degradation rates estimated by AOPWIN was discussed as well. Referring to QSAR forming the basis of AOPWIN, "it is expected that the predictions will be more uncertain the more complex the chemical is (i.e., how many functional groups it contains) and especially if the chemical contains halogen atoms and/or N- or S-atoms." (Atkinson et al. (1999)as cited in Muir et al. (2004)). This aspect may also be the case for more recent models.

Human Health

Current status of global regulation on Chlorpyrifos

18. On February 6, 2020, **Corteva**, formerly a part of Dow Dupont, announced the end of chlorpyrifos production by 2021 (Reuters, 2020). Corteva said this action is driven by declining demand. The company will continue to support chlorpyrifos in an EPA review.

19. On December 6, 2019, the **European Union** (EU) announced that it will no longer permit sales of chlorpyrifos after **January 31, 2020**. The Standing Committee on Plants, Animals, Food and Feed (PAFF Committee) voted in favor of two draft Implementing Regulations that denied the renewal of approvals for chlorpyrifos and chlorpyrifos-methyl. The European Commission adopted the Commission Implementing Regulation (EU) 2020/18 on 10 January 2020 (EC, 2020).

20. On October 9, 2019, California Department of Pesticide Regulation (**DPR**) announced, an agreement with pesticide manufacturers to end the sale of chlorpyrifos by **February 6, 2020 (CDPR, 2020a).**

21. On August 14, 2019, California Department of Pesticide Regulation (**DPR**) issued **cancellation notices** for pesticide products containing chlorpyrifos (CDPR, 2020b). The risk assessment that supports DPR's proposal to cancel chlorpyrifos products is based on five animal studies published in 2016, 2017, and 2018, that report neurotoxicity from chlorpyrifos at exposure levels that are considerably lower than the levels that cause acetylcholinesterase inhibition. Based on its **human health risk assessment**, DPR has concluded that developmental neurotoxicity is the critical endpoint for chlorpyrifos and has derived a point of departure for chlorpyrifos risk assessment. DPR presented its Toxic Air Contaminant (TAC) findings to California's Scientific Review Panel at a meeting on July 30, 2018, and the Panel subsequently concluded that the DPR assessment of the developmental neurotoxicity of chlorpyrifos was "based on sound scientific knowledge, and represents a balanced assessment of our current scientific understanding." (CalEPA, 2018).

22. On August 2, 2019, the European Food Safety Authority (**EFSA**) published a **human health risk** assessment for chlorpyrifos concluding that no safe exposure level could be determined for chlorpyrifos and that based upon available data, the approval criteria under Article 4 of Regulation (EC) No 1107/2009 for human health were not met (EFSA, 2019b). EFSA also published an updated **human health risk assessment for chlorpyrifos-methyl** on November 26, 2019, reiterating the same conclusion (EFSA, 2019a). EFSA's primary health concerns were potential developmental neurotoxicity based on the available animal data and epidemiological evidence, and unresolved concerns regarding potential genotoxicity.

23. In contrast, on July 18, 2019 **US EPA** issued a final order that denied all objections to the March 2017 petition denial order (US-EPA, 2019). EPA has stated that it intends to complete its evaluation of the epidemiology studies for chlorpyrifos, as well as the new animal data relied on by Californian EPA, in the context of the pending registration review of chlorpyrifos under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A final registration review decision concerning chlorpyrifos is due by **October 1, 2022**. EPA revised its **human health risk assessment for chlorpyrifos in 2016**, after receiving public comments on the 2014 risk assessment and feedback from the FIFRA Scientific Advisory Panel (US-EPA, 2016). EPA concluded that, despite several years of study, the science addressing neurodevelopmental effects remains unresolved and that further evaluation of the science is warranted regarding whether the potential exists for adverse neurodevelopmental effects to occur from current human exposures to chlorpyrifos.

Background

24. Organophosphate pesticides, like Chlorpyrifos, interfere with signaling from the neurotransmitter acetylcholine. This mechanism is well established for acute poisoning. It is also the primary insecticidal mechanism. There is significant evidence that Chlorpyrifos may affect a variety of neuronal targets and processes that are not directly related to the cholinergic enzyme acetylcholinesterase (AChE). These effects might occur at concentrations that did not significantly inhibit AChE activity.

25. Several enzymes play a role in the metabolism and toxicity of chlorpyrifos. In addition to inhibition of AChE, the chlorpyrifos oxon also binds to butyrylcholinesterase (BuChE) and irreversibly to carboxylesterases. The cytochrome P450 family of microsomal enzymes (CYPs) is responsible for its metabolic activation and deactivation. Isozymes involved in dearylation of chlorpyrifos are reported to be CYP2C19 and CYP3A4. The extent of and mechanisms for non-cholinesterase mechanisms remain to be fully characterized. Laboratory experiments in rats and cell cultures suggest that

exposure to low doses of chlorpyrifos may alter serotonin signaling and increase rat symptoms of depression; change the expression or activity of several serine hydrolase enzymes, including neuropathy target esterase and several endocannabinoid enzymes; and affect components of the cyclic AMP system (Casida et al., 2008; Connors et al., 2008; D. L. Eaton et al., 2008; Slotkin, 2004).

26. The enzyme paraoxonase 1 (PON1) detoxifies chlorpyrifos oxon, the more toxic metabolite of chlorpyrifos. In laboratory animals, PON1 protects against chlorpyrifos toxicity while individuals that do not produce PON1 are particularly susceptible (Costa et al., 2013). In humans, studies about the protective effect of PON1 activity on the toxicity of chlorpyrifos are mixed, with modest yet inconclusive evidence that higher levels of PON1 activity may protect against chlorpyrifos exposure. Human populations have genetic variation in PON1 that may influence the effectiveness at detoxifying chlorpyrifos oxon. Some evidence indicates that children born to women with low PON1 may be particularly susceptible to chlorpyrifos exposure. Further, infants produce low levels of PON1 until six months to several years after birth, likely increasing the risk from chlorpyrifos exposure early in life (Flaskos, 2012; Smith et al., 2014; Timofeeva & Levin, 2010).

Systemic toxicity

27. For humans, metabolic activation of chlorpyrifos occurs predominantly in the liver while detoxification can take place in the liver or plasma. Metabolism is generally rapid and extensive, with the parent and/or the principle metabolite, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), found only in trace concentrations in blood or urine. The biological half-life for the major metabolite in humans following oral or dermal exposure was approximately 27 hours and chlorpyrifos metabolites are excreted primarily in the urine (Nolan et al., 1984)

28. Chlorpyrifos was tested in four long term dietary studies in rat, mouse and dog. No evidence of repeated dose toxicity or carcinogenicity was observed, apart from significant decrease of RBC cholinesterase activity in the 2-year study in rats (supported by the 2-year study in dogs).

29. In an 18-month combined toxicity and carcinogenicity dietary study in mice Chlorpyrifos was administered at 0, 5, 50, 250 ppm (0, 1, 10, 50 mg/kg/d) in the diet (report #153115 as summarized in (Spain, 2019)). There were significant increases in absolute and relative adrenal weights of the top dose male. Lower doses were unaffected, and no increase was seen in females. Effects were seen in the livers of high dietary concentration males (hepatocyte fatty vacuolation) and pericholangitis in both sexes. Effects were seen in the eyes at the two highest dietary concentrations in males and the highest dietary concentration in females (all plausibly related to contact irritability of test article with resultant scratching). Histopathological examination did not reveal any statistically significant increase in the incidence of neoplasms in treated animals when compared with controls. There was no ChE NOEL in the tested dosage range (dose-related inhibition of plasma ChE in both sexes at weeks 42 and 78). Brain ChE was modestly reduced at 50 ppm and greatly reduced at 250 ppm (residual activity about 20% or less in both sexes and both sampling intervals). RBC AChE was reduced at 250 ppm only. There were no definitive cholinergic signs at any dose. The NOAEL was 5 ppm (1 mg/kg bw/day) on the basis of inhibition of erythrocyte and brain acetylcholinesterase activity

30. In two 2y combined toxicity and carcinogenicity dietary studies in rats (report #153114 as summarized in (Spain, 2019)), Chlorpyrifos was administered at 0, 0.05, 0.1, 1, 10 mg/kg/d and 0.01, 0.3, 6 mg/kg/d, respectively. Inhibition of cholinesterase activity was the main toxicological finding in both

studies. Plasma ChE levels were reduces ($p < 0.05$, two tailed) at day 44, but not at day 91 The lowest NOAELs was 0.1 mg/kg bw/day for inhibition of RBC ChE activity and 1 mg/kg bw/day for inhibition of brain acetylcholinesterase activity. At 10 mg/kg bw/day an increase in the size of adrenal gland was observed, which were characterised microscopically by increased vacuolization of the zona fasciculate (report #72300 as summarized in as cited in Spain (2019)). There was no increase in tumors of any type in any organ or tissue at any of the dose levels tested.

31. In a 2-year dog study (report #036338-036339 as summarized in Spain (2019)) was administered at 0, 0.01, 0.03, 0.1, 1, 3 mg/kg/d. The NOAEL for RBC ChE inhibition was 0.1 mg/kg bw/day. Aside from cholinesterase inhibition no other toxicological effect was detected in any of the dogs, including body weight, food consumption, haematological investigation, clinical chemistry or urinalysis. No test-material-related effects were seen on organ weights or histopathology.

Toxicity to reproduction

32. Reproductive toxicity of chlorpyrifos was assessed in two-generation and prenatal developmental toxicity studies in rat and rabbit. Apart from the separately discussed effects on developmental neurotoxicity (DNT), Chlorpyrifos showed no potential for impairment of reproductive outcome, fertility or teratogenicity:

33. In a two-generation reproductive study (report #097570 as summarized in Spain (2019)) the critical effect was the cholinesterase activity depression. When the brain AChE was severely depressed, parental generation showed decreased bodyweight gain and histopathological effects in adrenals. For pups, the only effect referred was a decreased pup bodyweight and reduced survival index. The parental NOAEL was 0.1 mg/kg bw/day, based on a statistical decrease in the RBC AChE activity, the offspring NOAEL was 1 mg/kg bw/day and the reproductive NOAEL was set at 5 mg/kg bw/day, the highest dose tested.

34. In a developmental study in rat (report #036344 as summarized in Spain (2019)), chlorpyrifos at concentrations of 0, 0.1, 3, 15 mg/kg/d did not induce teratogenicity. Maternal toxicity was noted at > 3 mg/kg bw/day by decreasing RBC cholinesterase activity, while at higher doses dams showed decreased bodyweight and clinical signs. Effects were evidenced by an increase in the post-implantation loss at maternal toxic doses (15 mg/kg bw/day). So, the rat maternal NOAEL was 0.1 mg/kg bw/day and the developmental NOAEL was 3 mg/kg bw/day.

35. In a developmental study in rabbits (report #153116 as summarized in Spain (2019)), chlorpyrifos at concentrations of 0, 1, 9, 81, 141 mg/kg/d did not induce teratogenicity. Maternal toxicity was evident at 141 mg/kg bw/day with decreased bodyweight gain and plasma cholinesterase activity inhibition, but no RBC/brain cholinesterase was evaluated. The developmental effects included decreased fetal growth and increased incidence of post-implantation loss at 140 mg/kg bw/day. Therefore, the maternal and developmental NOAEL for rabbit was 81 mg/kg bw/day.

36. In a prenatal developmental toxicity study in mice (report #036345 as summarized in Spain (2019)) chlorpyrifos at concentrations of 0, 1, 10, 25 mg/kg/d did not induce teratogenicity The critical effect was the inhibition of the RBC cholinesterase both in mothers and foetuses at 10 mg/kg bw/day. At higher doses (25 mg/kg bw/day), the mothers developed clinical signs, decreased bodyweight and food consumption and even deaths.

Foetuses decreased their fetal weight, the crown-rump length and there was an increased in the incidence of delayed ossification of the skull bones and unfused sternebrae. The maternal and developmental NOAEL for mice was 1 mg/kg bw/day.

Endocrine disruption

37. In July 2019, the European Food Safety Authority (EFSA, 2019b), published a statement on the available outcomes of the human health assessment in the context of the pesticides peer review of chlorpyrifos. The EFSA experts agreed that chlorpyrifos is not an ED in humans. The reasoning was that the overall dose–response pattern for cholinergic overstimulation indicates that chlorpyrifos is a potent AChE inhibitor, and this is practically limiting the possibility of exploring additional target organs/systems.

38. However, acetylcholine esterase inhibitors generally have capacity to inhibit p450 enzymes (i.e. CYP1A2 and CYP3A4), which are the major p450s that metabolize estradiol, estrone, and testosterone in the liver(C. T. Usmani KA, Rose RL, Hodgson E, 2006; R. R. Usmani KA, Hodgson E, 2003). By this mechanism, it is possible that exposure to these compounds can interfere with metabolism of hormones and, in so doing, disturb the normal hormonal balance.

Gentox

39. During the Pesticides Peer Review 01 Experts' meeting, the experts discussed the in vitro and in vivo regulatory studies provided in the RAR for chlorpyrifos (EFSA, 2019b). It was concluded that the results from six bacterial and three mammalian gene mutations assays overall showed that chlorpyrifos does not induce gene mutations in vitro.

40. Chlorpyrifos was also considered not capable to induce chromosome aberration in vitro. Four studies were considered, although three of them had some methodological limitations and therefore considered acceptable with reservations (one of these three studies produced positive findings), the fourth one was considered fully acceptable and provided negative results.

41. Six in vitro studies on unscheduled DNA synthesis: were submitted out of which two produced positive results; the two positive studies were considered acceptable as additional information and were retrieved from a well-documented publication (Cui et al., 2011).

42. Five in vivo studies in somatic cells (mouse bone marrow micronucleus test), although presenting some methodological limitations, consistently showed negative findings.

43. It was noted that several publications are available for chlorpyrifos which report chromosomal aberrations in vivo (Abdelaziz et al., 2010) and DNA damage in Comet assays both in vitro and in vivo (Cui et al., 2011; Kopjar et al., 2018; Mehta et al., 2008; Sandhu et al., 2013). Some of these publications present deficiencies but concerns observed in the public literature studies cannot be ignored and a genotoxic potential for chlorpyrifos cannot be ruled out.

44. EFSA (2019) noted that chlorpyrifos can produce DNA damage through topoisomerase II inhibition, as reported in one study using human foetal liver haematopoietic stem cells (Lu et al., 2015). Topoisomerase II inhibition may be involved as a molecular initiating event (MIE) for infant leukaemia (EFSA-PPRP et al., 2017).

45. The possible effects on haematopoietic stem cells have not been confirmed (Rodríguez-Cortez & Menéndez, 2020). This study analysed in vitro and in vivo the potential genotoxic effects of chlorpyrifos in human hematopoietic stem and progenitor cells (HSPC) at different ontogeny stages, spanning from embryonic to adult HSPCs, with a special emphasis in their ability to induce double-strand breaks (DSB) and Mixed-lineage leukemia (MLL) breaks. Chlorpyrifos induced MLL gene breaks (between 3% and 6%) in a dose independent manner regardless the cell type targeted. Chronic exposure of hESCs to Chlorpyrifos did not induce gross genomic instability measured by G-banding and DNA copy number variation profiling by CGH arrays. In vivo, long-term chronic exposure of NSG mice previously reconstituted with CB-derived CD34+ cells to Chlorpyrifos did not induce MLL breaks in the human graft Chlorpyrifos did not induce TOP2 poisoning even at high concentrations. The authors suggested that despite the ability of Chlorpyrifos to induce breaks in the hot spot region of MLL gene after a 24 hours hit, these compounds are not able to either induce detectable global DNA damage measured by gamma-H2AX levels neither to act as a TOP2 poisons. Furthermore, no MLL breaks were detected after chronic treatment in in vitro or in vivo systems, which indicates that lesions observed in single-pulse treatments are not enough to favour the enrichment of MLL-rearranged clones.

Ecotoxicology

Micro- and mesocosm studies on macroinvertebrates

46. In their paper, J. M. Giddings et al. (2014) reviewed existing micro- and mesocosm studies. Based on results from these experiments performed at community level but still in surrogate field conditions, the authors conclude that concentrations of 0.1 µg a.s/L or less cause no ecologically significant effects on aquatic communities. However, this conclusion should be mitigated. Indeed, the analysis of the authors is based on "no-observed-adverse-effect-concentration" (NOAEC), meaning that when observed, effects are followed by a recovery of the community. Recovery endpoints are however not suitable for all taxa. Compared to species with a very short generation time such as copepods (e.g. daphnids), macro-invertebrates and insects are mostly uni- and bivoltine species with only one or few generations per year. Accordingly, such organisms with less breeding cycles per year need a longer time to recover from a population collapse. Therefore, if the NOAEC (i.e. the recovery option) is deemed to be the relevant endpoint, such species with few generations per year must be imperatively included in the cosm experiment to achieve relevant conclusions for the whole aquatic community (i.e. species with short- and long-life cycle). Except for the Kansas outdoor cosms of (J. Giddings, 1993; J. M. Giddings et al., 1997) and Biever et al. (1994), Table 5 of J. M. Giddings et al. (2014) reports that no macroinvertebrates and/or insects were included in three out of the five other studies supporting a NOAEC of 0.1µg a.s./L (López-Mancisidor et al., 2008; Lopez-Mancisidor et al., 2008; Wijngaarden et al., 2005). On the opposite, in cosms where macroinvertebrates and/or insects were present, a NOAEC < 0.1µg a.s./L was determined in Brazner and Kline (1990), van den Brink et al. (1995) and Ward et al. (1995) (all references in Gidding et al., 2014). Finally, some other studies also reported in Table 5 of Giddings et al. (2014) clearly demonstrate that when included in micro- and mesocosms, taxa with no recolonization sources (such as insects, amphipods, and isopods) did not recover ((T. Brock et al., 1992; T. C. Brock et al., 2006) in J. M. Giddings et al. (2014)).

47. *Algae*

48. Although chlorpyrifos is not directly toxic for primary producers (E_bC_{50}= 460 µg a.s./L (m.m.) for *Selenastrum capricornutum*), several micro- and mesocosm experiments report important disequilibrium in the aquatic communities due to indirect effects. Because of the drastic decrease of the grazer invertebrates many authors observed a phytoplancton bloom within 6 to 8 weeks after application of chlorpyrifos (see references cited in Giddings et al. 2014 for the Dutch ditch cosms, California ponds and the Minnesota enclosures). Therefore, the occurrence of such indirect effects cannot be excluded in the field.

49. *Conclusions aquatic ecotoxicity*

50. Overall, laboratory studies clearly demonstrate that chlorpyrifos is highly toxic for aquatic communities at concentrations around 0.1 µg a.s./L and below for aquatic invertebrates. Whereas few model experiments performed with aquatic communities under more natural conditions (i.e. cosms) might support this value, other cosm studies clearly point out the ecological sensitivity of macroinvertebrates and insects when included in such modelled ecosystems. Based on the available data, threshold concentrations lower than 0.1 µg a.s./L can be expected for such sensitive taxa under natural conditions.

Field studies on aphids

51. In addition to laboratory tests, many field studies have been conducted with different formulations of Chlorpyrifos in various crops. These studies aim to identify potential long-lasting effects on arthropods communities.

52. As reported below, all full fauna field studies described in Spain (2017) demonstrate acute initial effects but also support the occurrence of long-lasting effects since no full recovery within the year following any application could be demonstrated for all taxa exposed. As an example, significant differences to the control plots in the abundance of predatory beetles of the family Staphylinidae were still identified one year after the application of a CS formulation of Chlorpyrifos to the tree canopy of apple orchards (see Bakker *et al*., (2007); Report No: 061012; (M279) in (Spain, 2017)). In a mature citrus plantation in Spain Aldershof et al. (2008) applied a water dispersible granules formulation of Chlorpyrifos (Dursban 75 WG) and reports that recovery within one year could not be probed for the coleopteran family Latridiidae. One year later, the population abundances of the hunting spider families Zodariidae, Gnaphosidae and Clubionidae were still lower than those of the control plots (see Report No: DA047FFC, 071032; (J164A) in (Spain, 2017)). When focusing on other soil macro- and micro-invertebrates, no full recovery in the year following the application of an EC formulation of Chlorpyrifos (Dursban 4 (EF-1042)) in a cereal field in England could be demonstrated for two Collembolan species *Lepidocyrtus cyaneus* and *Sminthurus viridis* (Report No: 040311; (J123A) in (Spain, 2017)).

Field studies on soil organisms

53. There are numerous field studies looking at the effects of Chlorpyrifos on soil macro-organisms communities (i.e. earthworms, mites and arthropods) are available. For e.g., when applied as EC formulation at a single rate of 480 g a.s./ha in permanent grassland in Germany and southern England, a full recovery of all these soil communities could not be observed within the year (see Mack (2012), Vaughan (2009) in (Spain, 2017). Similar conclusions

followed for apple Orchards in the UK treated yearly with an EC formulation (EF-1551) once before flowering (480 g a.s./ha) and once after flowering (960 g a.s./ha). This 2-year field monitoring recorded, among other, detrimental effects on the soil arthropods (especially on collembola) and earthworms (see Leopold (2015) in (Spain, 2017)).

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