STATEMENT

APPROVED: 31 July 2019

Statement on the available outcomes of the human health assessment in the context of the pesticides peer review of the active substance chlorpyrifos

European Food Safety Authority (EFSA)

Abstract

In July 2019, the European Commission asked EFSA to provide a statement on the available outcomes of the human health assessment in the context of the pesticides peer review for the renewal of approval of the active substance chlorpyrifos conducted in accordance with Commission Implementing Regulation (EC) No 844/2012. The current statement contains a summary of the main findings of the assessment related to human health following the pesticides peer review expert discussions in mammalian toxicology held between 1 and 5 April 2019, as well as EFSA's additional considerations, including whether the active substance can be expected to meet the approval criteria applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009. The identified concerns are presented as follows.

Keywords

chlorpyrifos, pesticide, insecticide, peer review, human health assessment

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Summary

Chlorpyrifos is an active substance covered by the third batch of the renewal programme for pesticides ('AIR3') in accordance with Commission Implementing Regulation (EU) No 844/2012.

Applications (June 2013) and supplementary dossiers (July 2015) for the renewal of approval of the active substance chlorpyrifos were submitted by a Task Force (comprising of Dow AgroSciences and Adama Agriculture B.V.) and by Sapec Agro SA.

An initial evaluation of the dossiers was provided by the rapporteur Member State (RMS) Spain in the Renewal Assessment Report (RAR) which was submitted to EFSA in July 2017. Subsequently, EFSA initiated a peer review of the pesticides risk assessment on the RMS evaluation in line with the provisions of Commission Implementing Regulation (EU) No 844/2012.

The commenting period was completed and included a public consultation on the RAR. Following evaluation of the comments received as well as the additional information provided by the applicants in response to a request in accordance with Article 13(3) of Regulation (EU) No 844/2012, a meeting of experts from EFSA and Member States, including relevant experts from the EFSA Panel on Plant Protection Products and their Residues (PPR Panel), took place to discuss certain elements related to mammalian toxicology.

In July 2019, prior to completion of the full peer review process, EFSA was mandated by the European Commission to provide a statement on the available outcomes of the human health assessment in the context of the peer review of chlorpyrifos.

The present statement contains a summary of the main findings of the assessment related to mammalian toxicology and human health following the Pesticides Peer Review Expert discussions in mammalian toxicology held between 1 and 5 April 2019. It also comprises EFSA's additional considerations, including whether the active substance can be expected to meet the approval criteria which are applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009.

Due to the fact that the genotoxic potential of chlorpyrifos remains unclear, toxicological reference values could not be established. Moreover, significant uncertainties were linked to the neurodevelopmental toxicity study, where effects were observed at the lowest dose tested in rats (decrease in cerebellum height corrected by brain weight). These concerns were supported by the available epidemiological evidence related to developmental neurological outcomes in children. In the absence of toxicological reference values, a risk assessment for consumers, operators, workers, bystanders and residents cannot be conducted. This issue represents a critical area of concern for chlorpyrifos.

In addition, the recorded toxicological effects meet the criteria for classification as toxic for reproduction category 1B (regarding developmental toxicity).

Based on the above results, it is considered that the approval criteria which are applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009 are not met.

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1 Introduction

Chlorpyrifos is an active substance covered by the third batch of the renewal programme for pesticides ('AIR3') in accordance with Commission Implementing Regulation (EU) No 844/2012[1].

Applications (June 2013) and supplementary dossiers (July 2015) for the renewal of approval of the active substance chlorpyrifos were submitted by a Task Force (comprising of Dow AgroSciences and Adama Agriculture B.V.) and by Sapec Agro SA. The rapporteur Member State (RMS) is Spain and the co-rapporteur Member State (co-RMS) is Poland.

An initial evaluation of the dossiers was provided by the RMS in the Renewal Assessment Report (RAR) which was submitted to EFSA on 3 July 2017 (Spain, 2017). On 18 October 2017, EFSA initiated a peer review of the pesticides risk assessment on the RMS evaluation, by dispatching the RAR to the Member States and applicants for consultation and comments in line with the provisions of Commission Implementing Regulation (EU) No 844/2012. In addition, a public consultation was also conducted.

After the completion of the commenting period, and following a comment evaluation phase, on 4 July 2018 EFSA requested the applicants to provide certain additional information related to all areas of the assessment including mammalian toxicology in accordance with Article 13(3) of Regulation (EU) No 844/2012which was evaluated by the RMS and presented in an updated RAR (Spain, 2019). Subsequently, in April 2019 a meeting of experts from EFSA and Member States including relevant experts from the EFSA PPR Panel took place to discuss certain elements related to mammalian toxicology.

By means of the mandate received on 1 July 2019 from the European Commission, prior to completion of the full peer review process, EFSA was requested to provide a statement with an overview of the available outcomes of the human health assessment in the context of the peer review of chlorpyrifos.

The present document is an EFSA statement containing a summary of the outcome of the expert consultation outlining the main findings of the assessment related to mammalian toxicology and human health following the pesticides peer review expert discussions in mammalian toxicology held in April 2019, including EFSA's additional considerations and an indication whether the active substance can be expected to meet the approval criteria which are applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009[2].

The list of end points for the active substance and the representative formulations assessed in the context of the peer review with regard to the impact on human health is available in Appendix A.

[1] Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26.

[2] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1.

1.1 Background and Terms of Reference as provided by the requestor

On 1 July 2019 EFSA was mandated by the European Commission to provide a statement with an overview of the available outcomes of the human health assessment in the context of the pesticides peer review for the renewal of approval of the active substance chlorpyrifos conducted in accordance with Commission Implementing Regulation (EU) No 844/2012.

In addition, EFSA was requested to indicate, whether the active substance chlorpyrifos can be expected to meet the approval criteria which are applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009.

2 Assessment

2.1 Mammalian toxicity

The toxicological profile of the active substance chlorpyrifos was discussed at the Pesticides Peer Review Experts' Meeting 01 in April 2019 and assessed based on the following guidance documents: SANCO/10597/2003-rev. 10.1 (European Commission, 2012), Guidance on dermal absorption (EFSA PPR Panel, 2012), ECHA/EFSA Guidance for the identification of endocrine disruptors (ECHA/EFSA, 2018) and Guidance on the application of the CLP Criteria (ECHA, 2017).

Regarding the technical specifications of the substance placed on the market by either of the three applicants, they are not supported by the toxicological assessment since the level of most impurities contained in the batches was not tested at adequate levels. However, regarding the toxicological relevance of the impurities, considering the toxicological profile including the high acute toxicity and the genotoxic potential of chlorpyrifos, it is not expected that the impurities present in the technical specification would have the potential to add additional hazard established for the parent. One impurity (sulfotep) has been considered as toxicologically relevant by the European Commission (European Commission, 2012). Its relevance is likely based upon the fact that it has a lower oral LD_{50} value than chlorpyrifos; no toxicological concern is identified for this impurity up to its specified limit in the technical specifications of 3 g/kg. The analytical methods used in the toxicological studies were not available for most of the toxicological studies, representing a concern in particular for the genotoxicity assessment (based on regulatory studies) but not for the critical findings which were retrieved from the published literature (such as the Columbia Center for Children's Environmental Health (CCCEH) study).

In rats, chlorpyrifos is extensively absorbed after oral administration, it is widely distributed, moderately to extensively metabolised by oxidation and hydrolysis, and eliminated mostly through urine within 48 h. An *in vitro* metabolism study indicates that liver microsomes from human, mouse and rat more readily produce a detoxication product (i.e. 3,5,6-trichloro-2-pyridinol – TCP) than an activation product (i.e. chlorpyrifos-oxon – CPO) and the formation of TCP has been estimated to exceed the formation of chlorpyrifos-oxon by a factor of 3. A data gap for the determination of the toxicokinetic values for chlorpyrifos (Tmax, Cmax, $t_{1/2}$, AUC) was identified.

In the acute toxicity studies, chlorpyrifos showed high, moderate and low acute toxicity when administered by the oral, dermal and inhalation routes respectively, meeting, in the view of the peer review experts, the classification criteria as Acute Tox. 3, H301 'Toxic if swallowed' and Acute Tox. 4, H312 'Harmful in contact with skin' according to the CLP criteria. It is noted that harmonised classification establishes only Acute Tox. 3, H301 according to Annex VI of Regulation (EC) No 1272/2008[3] regarding human health. The substance did not elicit a potential for skin or eye irritation, skin sensitisation or phototoxicity.

The main effect following short- to long-term repeated oral administration of chlorpyrifos was the inhibition of acetylcholinesterase (AChE) activity, which, at high dose levels, was leading to endogenous cholinergic overstimulation resulting in typical cholinergic symptoms. Erythrocyte (RBC) AChE inhibition was the critical effect in all studies. The relevant no-observed-adverse-effect level (NOAEL) was 0.1 mg/kg bw per day for both short-term and long-term exposure based on significant decrease of RBC AChE activity at 1 mg /kg bw per day in a 90-day and 2-year rat study supported by a 2-year study in dogs. No evidence for a carcinogenicity potential was found upon chlorpyrifos administration in rats or mice.

No information has been provided on the immunotoxic potential of chlorpyrifos, therefore a data gap was identified.

2.2 Genotoxicity

During the Pesticides Peer Review 01 Experts' meeting, the experts discussed the *in vitro* and *in vivo* regulatory studies provided in the RAR:

- gene mutation: the experts considered that the results from the six bacterial and the three mammalian gene mutations assays overall showed that chlorpyrifos does not induce gene mutations *in vitro*.
- chromosome aberration: chlorpyrifos was also considered not capable to induce chromosome aberration *in vitro*. Four studies were submitted: 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.
- unscheduled DNA synthesis: six *in vitro* studies 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).
- *in vivo* studies in somatic cells (mouse bone marrow micronucleus test): the five studies available in the dossiers and evaluated in the RAR, although presenting some methodological limitations, consistently showed negative findings.

The RMS proposed to the applicant to conduct a new *in vivo* Comet assay (according to OECD Test Guideline 489, OECD, 2014) with batches representative of the current production, in order to clarify the positive findings observed *in vitro* in one of the chromosome aberration

[3] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

tests and in two studies on unscheduled DNA synthesis. The applicants did not conduct and submit the new study during the renewal procedure. In addition, the experts noted that several publications are available for chlorpyrifos (some of them included in the RAR) which report chromosomal aberrations *in vivo* (Abdelaziz *et al.*, 2010) and DNA damage in Comet assays both *in vitro* and *in vivo* (Mehta *et al.*, 2008; Sandhu *et al.*, 2013; Kopjara *et al.*, 2018; Cui *et al.*, 2011). Although some of these publications present deficiencies as highlighted in the RAR, all the experts agreed that the concerns observed in the public literature studies cannot be ignored and that a genotoxic potential for chlorpyrifos cannot be ruled out. EFSA notes that other organophosphates (OPs) have been reported to cause DNA damage: chlorpyrifos and fenthion have been reported to induce oxidative stress resulting in tissue damage and nuclear DNA damage; diazinon has been shown to cause immediate and direct inhibitory actions on DNA synthesis (Adler *et. al.* 2006). Chlorpyrifos, methyl parathion and malathion have been reported to induce oxidative stress which, in turn, causes damage to all vital macromolecules including lipids, proteins and DNA: oxidative DNA damage can be followed by DNA single and double strand breaks; also, oxidative species may also interact with biological molecules to disrupt normal DNA synthesis and repair. Both acute and chronic exposure with chlorpyrifos, methyl parathion and malathion caused significantly marked DNA damage in rat tissues, namely liver, brain, kidney and spleen, when measured 24 hours post treatment (Ojha *et. al.* 2013).

It was also noted that chlorpyrifos can produce DNA damage through topoisomerase II inhibition, as reported in one study using human foetal liver hematopoietic stem cells (Lu *et al.*, 2015), which was mentioned in the EFSA Scientific Opinion on the "Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia" (EFSA PPR Panel, 2017), but not evaluated in the RAR. Topoisomerase II inhibition is a mechanism likely to have a threshold (EFSA Scientific Committee, 2011); in addition, topoisomerase II inhibition may be involved as a molecular initiating event (MIE) for infant leukaemia (EFSA PPR Panel, 2017). All the experts agreed that a new Comet assay study might not be able to cover this concern. Some experts also pointed out that epidemiological studies showed an important association between pesticides exposure and childhood leukaemia, including infant leukaemia (Ntzani, *et. al.* 2013; Hernández and Menéndez, 2016). It was noted that it is not possible to measure endpoints relevant for childhood leukaemia in current OECD standard Test Guidelines, due to higher sensitivity of hematopoietic stem and progenitor cells (HSPCs) compared to the standard cells, and the lack of exposure during the critical period (EFSA PPR Panel, 2017). This could be covered (in terms of exposure window, developmental period) by the extended one generation OECD 443 Test Guideline study (OECD, 2018), but the study is not designed for carcinogenicity assessment. Some experts indicated that this concern may be assessed by using a chromosome aberration study in HSPCs (because these cells have different sensitivity) by using the appropriate window of exposure. All the experts supported the RMS view on the need for additional data to address the concerns regarding chromosome aberration and DNA damage. However, they were not in a position to propose a specific study that could clarify all the above mentioned issues (chromosome aberration, DNA damage caused by oxidative stress or through topoisomerase II inhibition, infant leukaemia) and all the experts agreed that these uncertainties should be considered in the risk assessment.

2.3 Reproductive/developmental toxicity and endocrine disruption

In a two-generation reproductive toxicity study in rats, chlorpyrifos did not affect the reproductive performance up to the highest dose of 5 mg/kg bw per day tested, while RBC AChE inhibition was the critical effect related to parental toxicity with a NOAEL of 0.1 mg/kg bw per day; in this study, reduced pup growth and viability was observed with a NOAEL of 1 mg/kg bw per day. Developmental toxicity was investigated in rats, rabbits and mice. Rats were the most sensitive species in these studies. In rats, erythrocyte AChE inhibition was the critical effect identified regarding maternal toxicity, while increased post-implantation loss was seen at the highest dose tested. Decreased foetal size and increased post-implantation loss was observed in rabbits at maternal toxic doses (based on reduced body weight gain). No developmental toxicity potential was observed in mice.

The experts agreed that chlorpyrifos is not an endocrine disruptor (ED) in humans, because, in line with other ED assessments recently conducted by EFSA and the guidance for the identification of endocrine disruptors in the context of Regulation (EU) No 1107/2009 (ECHA/EFSA, 2018), an ED assessment is not scientifically necessary for chlorpyrifos. In all the studies conducted with chlorpyrifos, the NOAEL, the lowest-observable-adverse-effect level (LOAEL) and the maximum tolerated dose (MTD) were based on erythrocyte AChE inhibition and clinical signs at high doses. 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.

2.4 Developmental neurotoxicity (DNT)

During the Pesticides Peer Review 01 Experts' meeting in April 2019, Member State experts and two experts from EFSA's Panel on Plant Protection Products and their Residues (PPR Panel), discussed the available data regarding developmental neurotoxicity of chlorpyrifos. They took into consideration and discussed in details: a) an unpublished study in rats, 1998 (Spain, 2019); b) public literature presented in the systematic review provided by the applicants; c) additional literature provided by the experts or during the commenting period.

In the DNT study in rats (1998) (Spain, 2019), pregnant rats were exposed to different levels of chlorpyrifos (0.3, 1 and 5 mg/kg bw per day) from day 6 of gestation until postnatal day (PND) 11. This study was performed according to US EPA guideline OPPTS 870.6300 (US EPA, 1998) and presented some limitations according to the EPA guideline, as well as deviations from the current OECD 426 guideline (OECD, 2007) (lack of findings in the positive control, too short exposure period – from gestational day 6 to lactation day 11 instead of 21 –, lower number of individuals for neuropathology and for learning and memory, behavioural ontogeny, etc); however, the majority of experts agreed that the DNT effects observed in this study were relevant for the risk assessment. The results of the study indicated a decrease in body weight, food consumption and cholinergic toxicity in the dams at the highest dose level. In addition, a statistically significant dose-related decrease in plasma ChE and RBC AChE activities was observed in all treated groups; brain AChE activity was decreased at mid and high dose only. According to the contract laboratory, the relevant findings in pups (motor activity changes, decrease in body weight, etc.) were observed at the high dose level only. The RMS proposed a maternal LOAEL at 0.3 mg/kg bw per day, based on the inhibition of plasma ChE and RBC AChE, while a pup DNT NOAEL at 1 mg/kg bw per day, based on the decrease in body weight,

body weight gain and food consumption, decrease in the viability index, decrease in the absolute brain weight and increase in the relative brain weight observed at 5 mg/kg bw per day.

US EPA reviewed the same study in 2000 (US EPA, 2000) and concluded that: (1) there were adverse treatment related effects at 1.0 mg/kg bw per day (decrease in the measurement of the parietal cortex, supported by possible, although not significant, alterations in the hippocampal gyrus) in the brain of females at PND 66 and (2) a NOAEL could not be determined due to lack of morphometric data for low dose (0.3 mg/kg bw per day) and a LOAEL for the study was set by the US EPA at 0.3 mg/kg bw per day.

During the discussion of the findings of the DNT study during the peer review experts' meeting, particular attention was given to the re-evaluation of the study provided by Mie *et al.*, (2018). Mie expressed each brain regional measure relative to brain weight in order to properly demonstrate the absence of a sensitive target region: a statistically significant decrease in the cerebellum height corrected by brain weight was present in both sexes in the pups at 0.3 and 1 mg/kg bw per day. The absence of a statistically significant effect at high dose can be explained because the decrease of cerebellum height is paralleled with a significant decrease in brain weight (observed at the high dose only).

It is well known that morphometry of brain regions is a valuable data for regulatory authorities (Tsuji and Crofton, 2012): the decrease in cerebellum height corrected by brain weight was considered an adverse effect indicating a damage of the architecture of the developing brain (in 2014 the PPR Panel considered the relevance of morphometric analyses as endpoint for hazard characterisation[4]). The structural changes in the developing rat brain found in regulatory studies are consistent with human data. In particular, children with high prenatal exposure to chlorpyrifos showed frontal and parietal cortical thinning (Rauh *et al.*, 2012). During the peer review meeting, all the experts, but one, agreed to set the LOAEL of the study at 0.3 mg/kg bw per day (for both maternal and pup toxicity). The experts also considered that the reduction of cerebellum height corrected by brain weight could not be explained by the level of AChE inhibition at 0.3 and 1 mg/kg bw per day and this could be related to the difference in sensitivities to AChE inhibition in pups versus adult rats: foetuses are less exposed than dams and have a high rate of re-synthesis of foetal AChE that can result in less net inhibition of foetal AChE (Mattsson *et. al.,* 2000). The absence of the effect at high dose was considered related to the high maternal toxicity observed at the dose level tested.

The experts discussed other *in vivo*, *in vitro* evidence available from the public literature and the assessment performed in 2016 by US EPA (US EPA, 2016). They also discussed the potential key events of mode of action (MoA)/adverse outcome pathways (AOPs) for these developmental neurotoxicity effects: several publications indicate potential molecular initiating events (MIEs) or key events (KE) for DNT of chlorpyrifos and chlorpyrifos-oxon (e.g. inhibition of fatty acid amide hydroxylase (FAAH), decrease in calcium/calmodulin-dependent protein kinase type II (CaMKII), interference with tubulin polymerisation and axonal growth, axonal transport, etc.). The experts concluded that AOPs and MIEs for DNT cannot be described at this stage.

[4] https://www.efsa.europa.eu/sites/default/files/wgs/pesticides/wgDNTacetamipridimidacloprid.pdf

The experts discussed the epidemiological evidence showing associations between chlorpyrifos exposure during neurodevelopment and adverse health effects (attention deficit/hyperactivity disorders, decrease in intelligent quotient and working memory, etc). In particular, three main birth cohort studies were considered: the Columbia Center for Children's Environmental Health (CCCEH) study (US EPA, 2016), the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) (Castorina, *et. al.* 2010 and Marks *et. al.* 2010) and Mt. Sinai study (Sebe *et. al.,* 2005). Using different biomarkers of exposure, these studies show that prenatal exposure to OPs produces a consistent pattern of early cognitive and behavioral deficits (Rauh et al., 2012). The experts discussed also other epidemiological evidence from the public literature. The majority of the experts considered that the results from some of these studies (mainly from CCCEH study, Rauh *et al.*, 2012; Engel *et al.*, 2011; Silver *et al.*, 2017) contribute to the evidence of DNT effects in humans due to the exposure to chlorpyrifos and chlorpyrifos-methyl and occurring at doses lower than that causing 20% inhibition of AChE. Overall, separate lines of evidence indicate that chlorpyrifos and other OPs may affect a variety of neuronal targets and processes that are not directly related to AChE. Therefore, this would represent an additional concern to be taken into consideration for the risk assessment. In addition, it should be noted that in the CHAMACOS study measurement of trichloro-pyridinol (TCP) in urine[5], common metabolite of both chlorpyrifos and chlorpyrifos-methyl, contributed to the evidence of DNT effects in humans and exposure to chlorpyrifos and chlorpyrifos-methyl.

Taking into consideration the developmental neurotoxicity study outcome (reduction in cerebellum height – that could not be explained by the maternal AChE inhibition), the epidemiological evidence showing an association between chlorpyrifos exposure during development and neurodevelopmental outcomes, and the overall analysis of the published literature (*in vivo*, *in vitro* and human data), the experts suggested[6] that classification of chlorpyrifos as toxic for the reproduction, REPRO 1B, H360D 'May damage the unborn child' in accordance with the criteria set out in Regulation (EC) No 1272/2008 would be appropriate.

3 Conclusions

During the Pesticides Peer Review 01 Experts' meeting in April 2019, all the experts, except one, agreed that the Point of Departure (PoD) for chlorpyrifos should be the DNT LOAEL of 0.3 mg/kg. With regard to the uncertainty factors the experts went through the overall assessment and concluded that:

- the genotoxicity potential remains unclarified (positive findings from an *in vitro* chromosome aberration study and two *in vitro* unscheduled DNA synthesis assays; *in vivo* positive findings from open literature on chromosome aberration and on DNA damage caused through oxidative stress or by topoisomerase II inhibition which was considered a MIE for infant leukaemia);
- the effects recorded in the DNT study (decrease in cerebellum height corrected by brain weight already at the lowest dose tested, which is a relevant endpoint for hazard characterisation) indicate a concern;

[5] Post-meeting note: it is also possible that a significant portion of TCP present in urine samples can result from direct intake of TCP preformed in the environment and not as a result of chlorpyrifos or chlorpyrifos-methyl ingestion (Eaton et al., 2008).
[6] It should be noted that classification is formally proposed and decided in accordance with Regulation (EC) No 1272/2008.

- the epidemiological evidence supports the developmental neurological outcomes in children for chlorpyrifos

Overall, no reference values could in any case be set because of the unclear genotoxicity potential of chlorpyrifos; moreover, significant uncertainties were linked to the neurodevelopmental toxicity study, where effects were observed at the lowest dose tested in rats (decrease in cerebellum height corrected by brain weight). These concerns were supported by the available epidemiological evidence related to developmental neurological outcomes in children. In the absence of toxicological reference values, a risk assessment for consumers, operators, workers, bystanders and residents cannot be conducted. This issue represents a critical area of concern for chlorpyrifos.

In addition, the recorded toxicological effects meet the criteria for classification as toxic for reproduction category 1B (regarding developmental toxicity).

Based on the above it is considered that the approval criteria which are applicable to human health as laid down in Article 4 of Regulation (EC) No 1107/2009 are not met.

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Appendices

Appendix A - List of end points for the active substance and the representative formulations with regard to impact on human health

Impact on Human and Animal Health

Absorption, distribution, metabolism and excretion (toxicokinetics) (Regulation (EU) N° 283/2013, Annex Part A, point 5.1)

Rate and extent of oral absorption/systemic bioavailability	Rapid (84% - 93%) rats, based on urinary excretion
Toxicokinetics	Not available – data gap
Distribution	Widely distributed
Potential for bioaccumulation	No evidence for accumulation
Rate and extent of excretion	Nearly completely, excreted within 48 hours, mainly via urine (approx. 80%)
Metabolism in animals	Moderate-extensive. Steps: oxidation and hydrolysis
In vitro metabolism	The *in vitro* metabolic studies indicate that liver microsomes from human, mouse and rat more readily produce a detoxication product (i.e. 3,5,6,-trichloro-2-pyridinol – TCP) than an activation product (i.e. chlorpyrifos-oxon – CPO). These observations are similar to the *in vivo* metabolism studies in rodents.
Toxicologically relevant compounds (animals and plants)	Chlorpyrifos
Toxicologically relevant compounds (environment)	Chlorpyrifos

Acute toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.2)

Rat LD_{50} oral	66-223 mg/kg bw	H301
Rat LD_{50} dermal	1250-2000 mg/kg bw	H312
Rat LC_{50} inhalation	> 1.0 mg/L air per 4h (whole-body)	
Skin irritation	Non-irritant	
Eye irritation	Non-irritant	
Skin sensitisation	Non-sensitiser (M&K and Buehler tests)	

Phototoxicity	No phototoxicity potential	

Short-term toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.3)

Target organ / critical effect	Rat: Nervous system / RBC AChE inhibition Mouse: RBC and brain AChE inhibition Dog: RBC AChE inhibition	
Relevant oral NOAEL	90-day, rat: 0.1 mg/kg bw per day 90-day, mouse: 1 mg/kg bw per day 90-day & 2-year, dog: 0.1 mg/kg bw per day	
Relevant dermal NOAEL	21-day, rat: > 5 mg/kg bw per day	
Relevant inhalation NOAEL	14-day, rat: > 0.296 x 10^{-3} mg/L air (nose-only)	

Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)

In vitro studies	Bacterial gene mutation tests: 6 negative Mammalian gene mutation tests: 3 negative Chromosome aberration tests: - 2 negative (cultured rat lymphocytes and Chinese hamster ovary cells) - with some reservations - 1 positive (mouse spleen cells) - with some reservations - 1 negative (human peripheral blood lymphocytes) - acceptable UDS: Primary culture of rat hepatocytes: negative - with some reservations Rec-assay with *Bacillus subtilis*: negative - supportive Microtitration SOS chromotest: negative - supportive Sister chromatid exchange assay: negative - supportive with some reservations Cytokinetic and cytogenetic effect on human lymphoid cells: positive - supportive with some reservations	

	ICR mouse hepatocytes: dose-related increase in DNA damage (in the form of strand breaks) was seen in the comet assay, but UDS was not affected. DNA hypomethylation was seen at all concentrations - with some reservations	
In vivo studies	Micronucleus tests: - 3 negative (supportive with reservations) - 1 negative (supportive) - 1 negative (acceptable) DNA damage (mainly clastogenicity) reported in the public literature: - for chromosomal aberrations - for DNA damage in *in vivo* Comet assays	
Photomutagenicity	Not required	
Potential for genotoxicity	Chlorpyrifos did not induce gene mutation nor clastogenic effects in regulatory studies. Regarding DNA damage, positive results in Comet assay were observed *in vitro* and *in vivo* (well-documented publications). DNA damaging potential cannot be ruled out for chlorpyrifos.	

Long-term toxicity and carcinogenicity (Regulation (EU) N°283/2013, Annex Part A, point 5.5)

Long-term effects (target organ/critical effect)	Nervous system / RBC AChE inhibition (rat, mouse) Decrease in bw gain (rat)	
Relevant long-term NOAEL	0.1 mg/kg bw per day (2-years, rat) 0.9 mg/kg bw per day (18-month, mouse)	
Carcinogenicity (target organ, tumour type)	No carcinogenic potential	
Relevant NOAEL for carcinogenicity	10 mg/kg bw per day (highest dose tested in 2-year, rat studies) 47.1 mg/kg bw per day (highest dose tested in 18-month, mouse study)	

Reproductive toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.6)

Reproduction toxicity

Reproduction target / critical effect	Parental toxicity: RBC AChE inhibition Reproductive toxicity: no adverse effects Offspring's toxicity: Decreased pup growth and viability	
Relevant parental NOAEL	0.1 mg/kg bw per day	
Relevant reproductive NOAEL	5 mg/kg bw per day (highest dose tested)	
Relevant offspring NOAEL	1 mg/kg bw per day	

Developmental toxicity

Developmental target / critical effect	Rat: Maternal toxicity: RBC AChE inhibition Developmental toxicity: Increased post-implantation loss at maternal toxic doses Rabbit: Maternal toxicity: decreased bw gain Developmental toxicity: decreased foetal size and increased post-implantation loss Mouse: Maternal toxicity: RBC AChE inhibition Developmental toxicity: reduced AChE activity	
Relevant maternal NOAEL	Rat: 0.1 mg/kg bw per day Rabbit: 81 mg/kg bw per day Mouse: 1 mg/kg bw per day	
Relevant developmental NOAEL	Rat: 2.5 mg/kg bw per day Rabbit: 81 mg/kg bw per day Mouse: 1 mg/kg bw per day	

Neurotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.7)

Acute neurotoxicity	NOAEL= 10 mg/kg bw Clinical signs, decreased motor activity and grip performance, decreased bodyweight (between day 1-4 postdosing); AChE activity was not evaluated	

Repeated neurotoxicity	90-day, rat: NOAEL= 1 mg/kg bw per day Based on perineal soiling; AChE was not evaluated	
Additional studies (delayed neurotoxicity)	Acute & 90-day, hens: No evidence of delayed neurotoxicity	
Additional studies (developmental neurotoxicity)	Maternal LOAEL= 0.3 mg/kg bw per day, based on RBC AChE inhibition. Developmental neurotoxicity LOAEL= 0.3 mg/kg bw per day, based on reduction in cerebellum height – that could not be explained by the maternal AChE inhibition. Epidemiological evidence showed an association between chlorpyrifos exposure during development and neurodevelopmental outcomes. DNT potential of chlorpyrifos cannot be dismissed on the basis of the evaluation of the DNT studies provided in the RAR, the epidemiological evidence and analysis of the overall literature (*in vivo*, *in vitro* and human data).	H360D
Additional studies (AChE activity)	Critical effect: RBC AChE inhibition NOAEL acute = 1 mg/kg bw, rat NOAEL short-term = 0.1 mg/kg bw per day, rat	

Other toxicological studies (Regulation (EU) N° 283/2013, Annex Part A, point 5.8)

Supplementary studies on the active substance	• Acute oral study in humans: - LOAEL = 2 mg/kg bw per day based on RBC AChE inhibition (NOAEL = 1.0 mg/kg bw per day) • Subacute oral study in humans (males): - LOAEL= 0.5 mg/kg bw per day based on clinical symptoms • 6-week-dietary study in dogs: - peripheral tissue AChE inhibition NOAEL = 1 mg/kg bw per day - brain AChE inhibition NOAEL = 2 mg/kg bw per day; RBC AChE inhibition NOAEL < 0.5 mg/kg bw per day • Comparative Cholinesterase study in juvenile and preweaning adult rats after acute and repeated exposure to chlorpyrifos and chlorpyrifos-oxon (CCA study): - NOAEL in acute CCA study for RBC AChE inhibition: 0.5 mg/kg bw per day - NOAEL in repeated CCA study for RBC AChE inhibition: 0.1 mg/kg bw per day • Nose-only inhalation exposure to chlorpyrifos vapors (6h) in rats results in no clinical signs of exposure and no inhibition of AChE activity • The immunotoxic potential of chlorpyrifos could not be determined
Endocrine disrupting properties	An endocrine disruptor (ED) assessment in line with the current guidance for the identification of endocrine disruptors in the context of Regulation (EU) No 1107/2009 is not scientifically necessary for chlorpyrifos (ECHA/EFSA, 2018). In all the studies conducted with chlorpyrifos, the NOAEL, LOAEL and MTD were based on erythrocyte AChE inhibition and clinical signs at higher doses. 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.
Studies performed on metabolites or impurities	

3,5,6-trichloro-2-pyridinol (TCP)	• Rat oral LD_{50} is estimated in 3129 mg/kg bw in females • TCP did not show a genotoxic potential (Ames test, *in vitro* UDS and mammalian cell gene mutation, *in vivo* micronucleus). • 90-day, rat: NOAEL = 30 mg/kg bw per day based on ↑ liver and kidney weight • 1-year, dog: NOAEL = 12 mg/kg bw per day (based on based on ↓ body weight, haematological and clinical chemistry effects. • Developmental toxicity in rats: - Maternal NOAEL = 50 mg/kg bw per day based on ↓ in body weight gain - Developmental toxicity NOAEL = 150 mg/kg bw per day (highest dose tested) • Developmental toxicity in rabbit: - Maternal NOAEL = 100 mg/kg bw per day based on ↓ in body weight gain - Developmental toxicity NOAEL = 25 mg/kg bw per day based on ↑ incidence of foetal and litter CNS malformations • QSAR assessment: TCP is expected to be less toxic than chlorpyrifos **ADI = 0.06 mg/kg bw per day** (based on the NOAEL of 12 mg/kg bw per day from the 1-year study in dogs and applying an uncertainty factor of 200. **ARfD =0.25 mg/kg bw** (based on the NOAEL of 25 mg/kg bw per day from the rabbit developmental toxicity study and applying an uncertainty factor of 100.
2,3,5-trichloro-6-methoxypyridine (TMP)	• Rat oral LD_{50} > 2000 mg/kg bw in females • Three *in vitro* genotoxicity studies: negative (±S9) (Ames test, *in vitro* mammalian cells gene mutation and chromosome aberration assays)
3,6-dichloro-2-pyridinol (3,6-DCP)	• Rat oral LD_{50}: > 2000 < 5000 mg/kg bw (females) • Ames test (±S9): negative
Desethyl chlorpyrifos	• Rat oral LD_{50} cut-off value: 500 mg/kg bw (females) • Rat oral LD_{50} > 920 mg/kg bw (females) • Test Ames and *in vitro* micronucleus test: both negative • QSAR assessment: desethyl chlorpyrifos is expected to be less toxic than chlorpyrifos
Chlorpyrifos-oxon (CPO)	• Rat oral LD_{50} = 100/300 mg/kg bw (male and female, respectively) – Acute Tox. 3, H301 'Toxic if swallowed'

Medical data (Regulation (EU) N° 283/2013, Annex Part A, point 5.9)

No neurotoxic effects in manufacturing plant personnel reported. Evidence of polyneuropathy from acute poisonings. Epidemiological studies (taken together toxicity literature studies) suggest that chlorpyrifos might be acting on the developing nervous system through unknown mechanisms (H360D).

Summary[7] (Regulation (EU) N°1107/2009, Annex II, point 3.1 and 3.6)

	Value	Study	Uncertainty factor
Acceptable Daily Intake (ADI)	open(1, 2)	-	-
Acute Reference Dose (ARfD)	open(1, 2)	-	-
Acceptable Operator Exposure Level (AOEL)	open(1, 2)	-	-
Acute Acceptable Operator Exposure Level (AAOEL)	open(1)	-	-

(1) Reference values could not be derived since a genotoxic potential could not be excluded for chlorpyrifos

(2) Previously set toxicological reference values of chlorpyrifos (EFSA, 2014): ADI 0.001 mg/kg bw per day, AOEL 0.001 mg/kg bw per day, ARfD 0.005 mg/kg bw

Dermal absorption (Regulation (EU) N° 284/2013, Annex Part A, point 7.3)

Representative formulation (Pyrinex 250 CS, 250 g/L)	Concentrate: 25% Spray dilution (0.5 g/L): 70% Based on default values
Representative formulation (EF-1551 EC, 480 g/L)	Concentrate: 0.8% Spray dilution (1.8 g/L): 5% Spray dilution (0.48 g/L): 7% Based on triple pack approach
Representative formulation (RIMI 101 RB, 10 g/kg)	Concentrate: 9% Spray dilution: NA Based on *in vitro* study on human skin
Representative formulation (Chlorpyrifos-ethyl 5G GR, 50 g/kg)	Concentrate: - Spray dilution (0.351 g/L): 0.2% Based on *in vitro* study on human skin

[7] for metabolites, refer to section: Studies performed on metabolites or impurities

Representative formulation (SAP250 CS, 250 g/L)	Concentrate 25% Spray dilution: 70% Based on default values

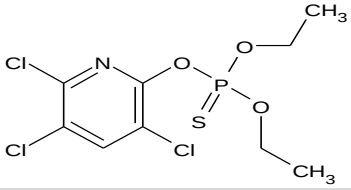
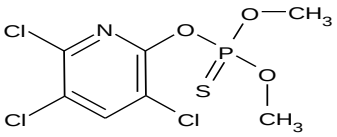
Exposure scenarios (Regulation (EU) N° 284/2013, Annex Part A, point 7.2)

Operators	Open. Risk assessment cannot be conducted in the absence of toxicological reference values.
Workers	Open. Risk assessment cannot be conducted in the absence of toxicological reference values.
Bystanders and residents	Open. Risk assessment cannot be conducted in the absence of toxicological reference values.

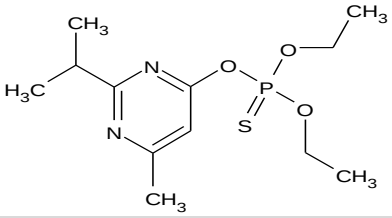
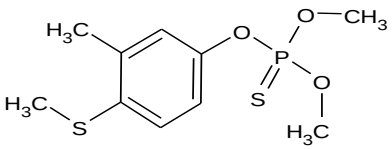
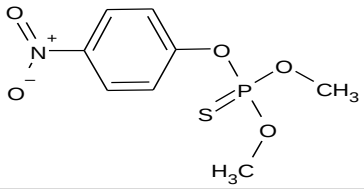
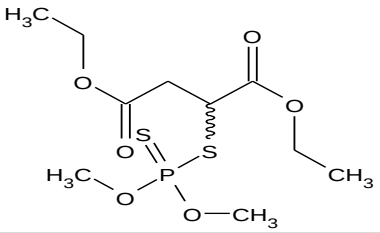
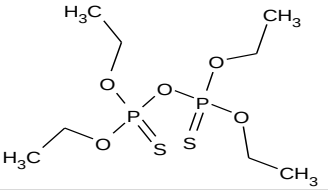
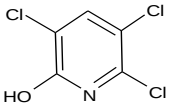
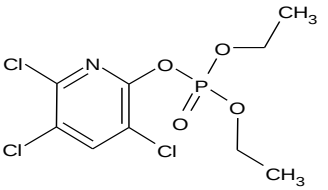
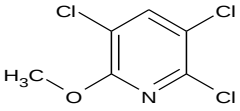
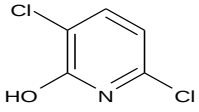
Classification with regard to toxicological data (Regulation (EU) N° 283/2013, Annex Part A, Section 10)

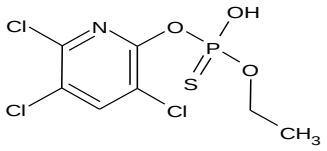
Substance:	Chlorpyrifos
Harmonised classification according to Regulation (EC) No 1272/2008 and its Adaptations to Technical Process [Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended][8] :	Acute Tox. 3, H301 'Toxic if swallowed'
Peer review proposal[9] for harmonised classification according to Regulation (EC) No 1272/2008:	Acute Tox. 3, H301 'Toxic if swallowed' Acute Tox. 4, H312 'Harmful in contact with skin' Repro 1B, H360D 'May damage the unborn child'

Appendix B - Used compound codes

Code/trivial name	IUPAC name/SMILES notation/InChIKey[(a)]	Structural formula[(b)]
chlorpyrifos	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate Clc1cc(Cl)c(Cl)nc1OP(=S)(OCC)OCC SBPBAQFWLVIOKP-UHFFFAOYSA-N	
chlorpyrifos-methyl	*O,O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate Clc1cc(Cl)c(Cl)nc1OP(=S)(OC)OC HRBKVYFZANMGRE-UHFFFAOYSA-N	

[8] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.
[9] It should be noted that classification is formally proposed and decided in accordance with Regulation (EC) No 1272/2008.

Code/trivial name	IUPAC name/SMILES notation/InChIKey[a]	Structural formula[b]
diazinon	*O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate Cc1cc(OP(=S)(OCC)OCC)nc(n1)C(C)C FHIVAFMUCKRCQO-UHFFFAOYSA-N	
fenthion	*O,O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate Cc1cc(ccc1SC)OP(=S)(OC)OC PNVJTZOFSHSLTO-UHFFFAOYSA-N	
parathion-methyl	*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate S=P(Oc1ccc(cc1)[N+]([O-])=O)(OC)OC RLBIQVVOMOPOHC-UHFFFAOYSA-N	
malathion	*S*-1,2-bis(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate CCOC(=O)CC(SP(=S)(OC)OC)C(=O)OCC JXSJBGJIGXNWCI-UHFFFAOYSA-N	
sulfotep	*O,O,O′,O′*-tetraethyl dithiopyrophosphate CCOP(=S)(OCC)OP(=S)(OCC)OCC XIUROWKZWPIAIB-UHFFFAOYSA-N	
TCP	3,5,6-trichloro-2-pyridinol Clc1cc(Cl)c(Cl)nc1O WCYYAQFQZQEUEN-UHFFFAOYSA-N	
chlorpyrifos-oxon (CPO)	diethyl 3,5,6-trichloro-2-pyridyl phosphate Clc1cc(Cl)c(Cl)nc1OP(=O)(OCC)OCC OTMOUPHCTWPNSL-UHFFFAOYSA-N	
TMP	2,3,5-trichloro-6-methoxypyridine Clc1cc(Cl)c(Cl)nc1OC RLIVUWLXZBDMBL-UHFFFAOYSA-N	
3,6-DCP	3,6-dichloro-2-pyridinol Oc1nc(Cl)ccc1Cl UGPDKBDRRLFGFD-UHFFFAOYSA-N	

Code/trivial name	IUPAC name/SMILES notation/InChIKey[a]	Structural formula[b]
desethyl chlorpyrifos	*O*-ethyl *O*-(3,5,6-trichloro-2-pyridyl) hydrogen (*RS*)-phosphorothioate Clc1cc(Cl)c(Cl)nc1OP(O)(=S)OCC WHGNMEMHTPXJRR-UHFFFAOYSA-N	

(a): ACD/Name 2018.2.2 ACD/Labs 2018 Release (File version N50E41, Build 103230, 21 Jul 2018)
(b): ACD/ChemSketch 2018.2.2 ACD/Labs 2018 Release (File version C60H41, Build 106041, 07 Dec 2018)

Glossary and abbreviations

a.s.	active substance
AChE	acetylcholinesterase
ADI	acceptable daily intake
AAOEL	acute acceptable operator exposure level
AOEL	acceptable operator exposure level
AOP	adverse outcome pathway
ARfD	acute reference dose
AUC	area under the blood concentration/time curve
bw	body weight
CaMKII	calcium/calmodulin-dependent protein kinase type II
CCCEH	Columbia Center for Children's Environmental Health
CHAMACOS	Center for the Health Assessment of Mothers and Children of Salinas
ChE	cholinesterase
CLP	classification, labelling and packaging
Cmax	concentration achieved at peak blood level
CNS	central nervous system
CPF	chlorpyrifos
DNA	deoxyribonucleic acid
DNT	developmental neurotoxicity
ECHA	European Chemicals Agency
ECHA RAC	European Chemicals Agency, Risk Assessment Committee
EC	European Commission
ED	endocrine disruption
EU	European Union
FAAH	fatty acid amide hydroxylase
FOB	functional observation battery
Hb	haemoglobin
Hct	haematocrit
HSPC	hematopoietic stem and progenitor cells
ICR	Institute of Cancer Research
KE	key event
LC_{50}	lethal concentration, median
LD_{50}	lethal dose, median; dosis letalis media
LOAEL	lowest observable adverse effect level
MIE	molecular initiating event
M&K	Maximization test of Magnussen and Kligman
MoA	mode of action

MS	Member State
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OP	organophosphate
PND	post-natal day
PoD	point of departure
ppb	parts per billion (10^{-9})
ppm	parts per million (10^{-6})
PPR panel	EFSA's Panel on Plant Protection Products and their Residues
QSAR	quantitative structure–activity relationship
RAR	Renewal Assessment Report
RBC	red blood cells
RMS	rapporteur Member State
SD	standard deviation
$t_{1/2}$	half-life (define method of estimation)
Tmax	time until peak blood levels achieved
TSH	thyroid-stimulating hormone (thyrotropin)
UDS	unscheduled DNA synthesis
US EPA	United States Environmental Agency
UF	uncertainty factor
WHO	World Health Organization