

Reconsideration of the human toxicological reference values (ARfD, ADI) for chlorpyrifos

BfR opinion No 026/2012, 1 June 2012

The insecticide chlorpyrifos has been tested in numerous toxicological studies in the past. On the basis of these studies, the EU-evaluation of the active ingredient was finalized in 2005. Since then, new studies and data on the toxicity of chlorpyrifos have been published. The US American Environmental Protection Agency EPA has evaluated these studies and derived new toxicological reference values. The BfR has come to the conclusion that within the EU too, a reassessment of the human health risks associated with chlorpyrifos should be conducted.

Chlorpyrifos is a comprehensively studied pesticide for which an expansive suite of toxicity tests exists. New research on chlorpyrifos has been reported and published, since the EU evaluation was finalised in 2005.

In summer 2011, the US EPA released a preliminary human health risk assessment that focused on the AChE inhibiting potential of chlorpyrifos (US EPA, 2011a). A full weight of the evidence including characterization of uncertainty has not yet been finalised (see Annex).

The new toxicological studies, mechanistic data, epidemiologic and biomonitoring studies submitted to the US EPA and the new information provided in the literature seem to support that also in the EU a reconsideration and Peer-Review of threshold values should be initiated followed by a risk assessment based on actual data on consumer exposure as well as occupational exposure.

Acute Reference Dose (ARfD)

For chlorpyrifos, the EU (2005) has established an ARfD of 0.1 mg/kg bw on the basis of a NOAEL of 10 mg/kg bw from "acute and delayed neurotoxicity studies in rats" as a Point of Departure (PoD) and a safety factor of 100.

In 2011, the US EPA has received and reviewed a new toxicological study on chlorpyrifos (Marty & Andrus, 2010) in which acetylcholinesterase (AChE) inhibition in postnatal day (PND) 11 and adult rats has been measured after acute and repeated exposure. The main results of this study have also been published recently (Marty et al., 2012). With acute exposure, the AChE inhibition NOAELs were 2 mg/kg bw for brain and 0.5 mg/kg bw for red blood cells (RBCs) in both age groups. In addition, a benchmark dose (BMD) analysis including a BMD meta-analysis of the combined pup and adult dataset was performed (Reiss et al, 2012). For acute doses, the meta-analysis resulted for both age groups in a BMD₂₀ for RBC AChE of 1.0 mg/kg bw with a BMDL₂₀ of 0.90 mg/kg bw, and a BMD₁₀ for brain AChE of 1.7 mg/kg bw with a BMDL₁₀ of 1.3 mg/kg bw for brain AChE.

Based on the new data mentioned above (Marty & Andrus, 2010) and a further study in male PND 17 rats (Moser et al., 2006), the US EPA (2011) concluded that male and female pup RBC ChE and male whole blood ChE inhibition were the most sensitive endpoints and appropriate as a PoD for the acute dietary exposure scenario. A BMDL₁₀ of 0.36 mg/kg bw associated with RBC ChE inhibition in male and female rat pups exposed to chlorpyrifos in milk (Marty & Andrus, 2010) was selected as a suitable PoD with support from the BMDL₁₀ of 0.4 mg/kg from Moser *et al.* (2006). Using an uncertainty factor of 100, an acute population adjusted dose (aPAD) of 0.0036 mg/kg bw was derived by the US EPA.

Acceptable Daily Intake (ADI)

For chlorpyrifos, the EU (2005) has established an ADI of 0.01 mg/kg bw on the basis of a NOAEL of 1 mg/kg bw from "2-years rats, mice and dogs" studies as a Point of Departure (PoD) and a safety factor of 100.

In the new toxicological study on chlorpyrifos (Marty & Andrus, 2010; Marty et al., 2012), for repeated exposures, the AChE inhibition NOAELs were 0.5 mg/kg bw for brain and 0.1 mg/kg bw for red blood cells (RBCs) in immature (PND 11) and adult rats. In addition, a benchmark dose (BMD) analysis including a BMD meta-analysis of the combined pup and adult dataset was performed (Reiss et al, 2012). For repeated doses, the meta-analysis resulted for both age groups in a BMD₂₀ for RBC AChE of 0.21 mg/kg bw with a BMDL₂₀ of 0.19 mg/kg bw, and a BMD₁₀ for brain AChE of 0.67 mg/kg bw with a BMDL₁₀ of 0.53 mg/kg bw for brain AChE.

For the chronic dietary risk assessment, the US EPA did not use the results from the new toxicological study (Marty & Andrus, 2010) but the results of an earlier developmental neurotoxicity study (Hoberman, 1998 a,b) from which a BMDL₁₀ of 0.03 mg/kg bw for RBC ChE inhibition in pregnant rats was selected as a suitable chronic PoD. Using an uncertainty factor of 100, a chronic population adjusted dose (cPAD) of 0.0003 mg/kg bw was derived by the US EPA.

Conclusion

The new toxicological studies, mechanistic data, epidemiologic and biomonitoring studies submitted to the US EPA and the new information provided in the literature seem to support NOAELs for acute AChE inhibition of 2 mg/kg bw (brain) and 0.5 mg/kg bw (RBCs) and NOAELs for AChE inhibition after repeated exposure of 0.5 mg/kg bw (brain) and 0.1 mg/kg bw (RBCs).

Therefore, the NOAEL of 10 mg/kg bw which was used for setting the ARfD in the EU can not any longer be considered to be an appropriate PoD for acute human risk assessment, especially for the protection of infants and children. Consequently, the ARfD (0.1 mg/kg bw) which has been established in the EU in 2005 should be reconsidered.

Furthermore, both the NOAEL of 1 mg/kg bw which was used as PoD for chronic human risk assessment and the ADI (0.01 mg/kg bw) which has been set in the EU in 2005 should also be reconsidered.

Also, the NOAEL of 1 mg/kg bw which was used for setting the AOEL in the EU can not any longer be considered to be an appropriate PoD for occupational and residential human risk assessment, especially for the acute effects which could require the derivation of an Acute AOEL. Consequently, the AOEL (0.01 mg/kg bw) which has been established in the EU in 2005 should also be reconsidered.

After reconsideration and Peer-Review of the above mentioned reference values in the EU, a risk assessment should be performed based on actual data on consumer exposure as well as occupational exposure of all authorised uses of pesticides in the European countries including the conclusions from the new toxicological studies, mechanistic data, epidemiologic and biomonitoring studies submitted to the US EPA.

References

- EU (2005) Review report for the active substance chlorpyrifos. SANCO/3059/99 – rev. 1.5, 3 June 2005.
- Hoberman AM (1998 a,b) Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, Pennsylvania (Laboratory Study No. 304-001).
- Marty MS, Andrus MK (2010) Comparison of cholinesterase (ChE) inhibition in young adult and preweanling CD rats after acute and repeated chlorpyrifos exposures. Toxicology & Environmental Research and Consulting, The Dow Chemical Company. Midland, Michigan 48674. Study ID: 091107. MRID No.: 48139301, Unpublished.
- Marty MS, Andrus AK, Bell MP, Passage JK, Perala AW, Brzak KA, Bartels MJ, Beck MJ D.R. Juberg (2012) Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos-oxon. *Regulatory Toxicology and Pharmacology* 63, 209–224.
- Moser VC, Simmons JE, Gennings C (2006) Neurotoxicological interactions of a five-pesticide mixture in preweanling rats. *Toxicol Sci* 92, 235-45.
- Reiss R, Neal B, Lamb JC, Juberg DR (2012) Acetylcholinesterase inhibition dose–response modeling for chlorpyrifos and chlorpyrifos-oxon. *Regulatory Toxicology and Pharmacology* 63, 124-131.
- US EPA (2011a) Chlorpyrifos: Preliminary human health risk assessment for registration review. Date: 30.06.2011.

Annex:**Summary and conclusions from the FIFRA Draft Issue Paper of the Scientific Advisory Panel in April 2012**

Chlorpyrifos is a comprehensively studied pesticide using an expansive suite of toxicity tests conducted in accordance with harmonized, scientifically peer-reviewed study protocols and published in the scientific literature. New research on chlorpyrifos, including epidemiological studies in mothers and children, has posed the issue of whether AChE inhibition is the most sensitive endpoint, and thus have raised some uncertainty in the chlorpyrifos risk assessment. In order to determine the degree to which these recent studies are appropriate for incorporation into risk assessment the US EPA is taking a stepwise approach to evaluate and interpret all the scientific information related to the potential for adverse neurodevelopmental effects in infants and children as a result of prenatal exposure to chlorpyrifos, as well as to characterize thoroughly the strengths and uncertainties associated with these studies (FIFRA SAP, 2012).

The evaluation of these recent studies began in September 2008 and subsequent Agency activities have involved developing the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for integration of epidemiology with other types of experimental data (USEPA, 2010; FIFRA SAP 2010). In summer 2011, the Agency released its preliminary human health risk assessment that focused on the AChE inhibiting potential of chlorpyrifos (US EPA, 2011a). Additionally an Occupational and Residential Exposure Assessment was also published (US EPA, 2011b).

A full weight of the evidence including characterization of uncertainty has not yet been conducted; however, the April 2012 FIFRA Draft Issue Paper of the Scientific Advisory Panel (FIFRA SAP, 2011, 2012) is an important step toward this effort. The types of information reviewed by FIFRA SAP (2012) are less often available and therefore approaches for their use in risk assessment (qualitatively or quantitatively) are less well established (e.g., epidemiology studies, biomonitoring data, modes of action/adverse outcome pathways for neurodevelopmental outcomes, etc). Each of these lines of evidence is important for the pending weight of the evidence analysis and uncertainty characterization.

- *AChE inhibition:*
Consistent with the concept that younger juvenile animals are more sensitive due to immature metabolic systems, PND11 rat pups provide the most sensitive cholinesterase inhibition data for deriving the acute oral PoD (BMDL10 0.36 mg/kg). In repeated dosing studies, pups exhibit similar sensitivity to adult rats and it is pregnant dams which provide the most sensitive data for deriving a repeated exposure oral point of departure (BMDL10 0.03 mg/kg/d).
- *Neurodevelopmental Outcomes in Laboratory Animals:*
Many studies report effects at a dose of 1 mg/kg/d, a dose that produces some amount of brain ChE inhibition when given directly to the pups postnatally, but may or may not alter fetal brain ChE activity when given to the dams gestationally. One study (Braquenier et al., 2010) using lower doses, administered to the dam on GD15-LD14, reported a NOEL of 0.2 mg/kg/d. These new studies suggest a potential for long-term effects of chlorpyrifos from early-life exposure and they suggest neurodevelopmental effects below doses which elicit 10% AChE inhibition.

- *Epidemiology studies in mothers and children:*
US EPA summarized the available epidemiologic data concerning the relation between gestational exposure to chlorpyrifos and adverse neurodevelopmental outcomes in infants and children. The epidemiology studies reviewed are well-conducted studies with numerous strengths, as well as some limitations. These limitations include use of non-specific biomarkers of chlorpyrifos exposure in some of these studies, lack of measurement of postnatal chemical exposure, and the proportion of missing data among several key variables in these investigations. In occupational settings, exposure measurement error has been shown to more greatly influence epidemiology study results than unknown or unmeasured confounding variables (Blair et al., 2011). For example, Rauh et al. (2011) reported that, in the Columbia cohort, “the dose-effect relationships between CPF [chlorpyrifos] exposure and log-transformed Working Memory Index and Full-Scale IQ scores are linear across the range of exposures in the study population, with no evidence for a threshold” (Rauh et al., 2011). Departures from linearity were not statistically significant. Two possible explanations for this finding are that 1) the exposure response is linear on the scale assessed, or that 2) the studies did not have sufficient power to detect departures from linearity in the shape exposure-outcome relationship. Overall, the newly available data support and strengthen the conclusion that prenatal exposure to chlorpyrifos likely plays a role in adverse neurodevelopmental outcomes measured in children.
- *Biomonitoring data:*
Chlorpyrifos is one of a small set of pesticides for which there is a large database of biomonitoring studies available for a variety of lifestages and subpopulations. Biomonitoring data provide real world information on exposed individuals; however, their interpretation is challenging given that the timing, magnitude, and source of exposures are most often unknown or only partially known. At this time, the existing models are not equipped for a sophisticated analysis. It is proposed that this is an area where additional research could significantly improve the ability to determine whether actual exposures experienced by participants in the epidemiology studies were above or below AChE inhibition concentrations.

With respect to the key questions being considered by the US EPA in 2012 (*i.e.*, whether chlorpyrifos causes long-term effects from fetal or early life exposure and if adverse effects can be attributed to doses lower than those which elicit 10% inhibition of AChE), when taken together the evidence from the experimental toxicology studies evaluating outcomes such as behavior and cognitive function; mechanistic data on possible adverse outcome pathways/modes of action; and epidemiologic and biomonitoring studies:

- Qualitatively, US EPA preliminarily concludes that these lines of evidence together support a conclusion that exposure to chlorpyrifos results in adverse neurodevelopmental outcomes in humans, at least under some (still unclear) conditions.
- Quantitatively, the dose –response relationship of AChE inhibition across different life stages is established, but other adverse outcome pathways/modes of action are not established. The question posed regards the nature and degree of uncertainty around points of departure based on 10% AChE inhibition to protect against neurodevelopmental outcomes.

Additional References to the Annex

- Blair, A., Thomas, K., Coble, J., Sandler, D. P., Hines, C. J., Lynch, C. F., et al. (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med*.
- Braquenier, J. B., Quertemont, E., Tirelli, E., & Plumier, J. C. (2010). Anxiety in adult female mice following perinatal exposure to chlorpyrifos. *Neurotoxicol Teratol*, 32(2), 234-239.
- FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.
- FIFRA Scientific Advisory Panel. (2011). Chlorpyrifos Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK-PD) Modeling linked to Cumulative and Aggregate Risk Evaluation System (CARES). Report from the FIFRA Scientific Advisory Panel Meeting of February 15-18, 2011. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: <http://www.epa.gov/scipoly/sap/meetings/2011/index.html>.
- FIFRA Scientific Advisory Panel. (2012). “ **Draft Issue Paper: Scientific Issues Concerning Health Effects of Chlorpyrifos April 10-13, 2012** Available: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0040-0002>.
- Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect*, 119(8), 1196-1201.
- Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics*, 118(6), e1845-1859.
- Ray, A., Liu, J., Ayoubi, P., & Pope, C. (2010). Dose-related gene expression changes in forebrain following acute, low-level chlorpyrifos exposure in neonatal rats. *Toxicol Appl Pharmacol*, 248(2), 144-155.
- U.S. Environmental Protection Agency. (2010a). Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.
- US EPA (2011a) Chlorpyrifos: Preliminary human health risk assessment for registration review. Date: 30.06.2011.
- U.S. Environmental Protection Agency. (2011b). Chlorpyrifos: Occupational and Residential Exposure Assessment, EPA Barcode: D388165. In Office of Pesticide Programs (Ed.). Washington D.C.