



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

JUL 15 2014

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Re: Chlorpyrifos petition dated September 12, 2007; July 2014 Response

Dear Mr. Colangelo and Dr. Reeves:

I am writing to further respond and update you on the U.S. Environmental Protection Agency's (EPA) continued efforts to address the Natural Resources Defense Council (NRDC) and Pesticide Action Network North America (PANNA) jointly submitted September 12, 2007¹ petition, and our related efforts to complete the registration review of chlorpyrifos. The petition specifically requested that EPA revoke all tolerances and cancel all registrations for the insecticide chlorpyrifos.

We have made significant progress in completing our work on the remaining four petition issues that we did not address in our July 16, 2012² partial response to your petition. Today we are responding to your claim that EPA failed to incorporate inhalation routes of exposure from chlorpyrifos volatilization. This will complete our response to your broader claim that EPA failed to incorporate inhalation routes of exposure since we have already addressed the primary spray drift portion of this claim in our July 16, 2012 response. At that time, as you may recall, in conjunction with our partial petition response, we released our *Evaluation of the Potential Risks From Spray Drift and the Impacts of Potential Risk Reduction Measures*³ along with spray drift mitigation that the chlorpyrifos registrants agreed to implement. The label mitigation (in the form of rate reductions and spray drift buffers) reduces risks to bystanders, particularly children, from

¹ Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2007-1005-0005>.

² Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2007-1005-0095>.

³ Available at <http://www.regulations.gov/#!docketDetail;D=EPA-HQ-OPP-2008-0850>.

spray drift. We approved those changes for all 41 chlorpyrifos agricultural products subject to these use limitations by the end of 2012.

At this time, work still remains to be completed in order to respond to the other three remaining claims in your petition: (1) EPA failed to incorporate into its risk assessment, in a quantitative manner, data indicating that long-lasting effects in children result from early life exposure to chlorpyrifos; (2) EPA disregarded data demonstrating that there is no evidence of a safe level of exposure during pre-birth and early life stages; and (3) EPA failed to cite or quantitatively incorporate studies and clinical reports suggesting potential adverse effects below 10% cholinesterase inhibition. In addition to addressing your claim regarding chlorpyrifos volatilization, our response today will outline the approach we are taking for completing our work on those remaining petition issues and our anticipated schedule for releasing a revised human health risk assessment (RHHRA) for public comment, which informs our petition response on the remaining three issues. The RHHRA will also address risks from human dietary exposure resulting from drinking water. Although drinking water exposures were not a part of your petition, we believe that for purposes of efficiency and completeness, our work on the RHHRA, which is currently scheduled to be issued for comment in December 2014 and our response to your petition (i.e., either a proposed revocation rule or a proposed order denying your petition) will be on a similar schedule.

Inhalation Exposure from Volatilization

As you are aware, in February 2013 we released our *Chlorpyrifos: Preliminary Evaluation of the Potential Risks from Volatilization*⁴ for chlorpyrifos for public comment. The assessment evaluated the potential risks to bystanders, those who live and/or work in proximity to treated fields, from inhalation exposure to vapor phase chlorpyrifos and chlorpyrifos-oxon emitted from fields following application of chlorpyrifos. The assessment was significant because it was the first probabilistic assessment of the risks posed by the post-application volatilization of a semi-volatile pesticide. The methodology used reflected the approach we employed for the fumigant pesticides where we assessed bystander inhalation risks from volatilization, the key exposure pathway for fumigants.⁵ In addition, it was consistent with the recommendations from the December 2009 Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP)⁶ meeting on the scientific issues associated with field volatilization of conventional (semi-volatile) pesticides. It also is similar to the methodology that was used to develop our recent Draft Human Health Bystander Screening Level Analysis: Volatilization of Conventional Pesticides that was released for public comment on March 28, 2014.⁷

⁴ R. Bohaty, C. Peck, A. Lowit, W. Britton, N. Mallampalli, and A. Grube. Chlorpyrifos: Preliminary Evaluation of the Potential Risks from Volatilization. 1/31/2013. U.S. EPA Office of Chemical Safety and Pollution Prevention. Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0114>.

⁵ The assessments can be found in the dockets for each fumigant. Four of which are provided here chloropicrin - EPA-HQ-OPP-2007-0350; dazomet - EPA-HQ-OPP-2005-0128; metam sodium/potassium - EPA-HQ-OPP-2005-0125; and methyl bromide - EPA-HQ-OPP-2005-0123.

⁶ U.S. EPA 2009. FIFRA Science Advisory Panel Meeting Minutes - Scientific Issues Associated with Field Volatilization of Conventional Pesticides. Available at <http://www.epa.gov/scipoly/sap/meetings/2009/december/120309meetingminutes.pdf>.

⁷ Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2014-0219-0002>.

The results of the preliminary assessment released in February 2013 indicated that offsite concentrations of volatilizing chlorpyrifos and chlorpyrifos-oxon may exceed the target concentration based on the toxicological endpoints used. It is important to understand, however, that the chlorpyrifos air concentration used in that assessment was derived from a study⁸ that measured the effects of aerosolized chlorpyrifos, which is the form chlorpyrifos takes when applied as a spray – not the form it takes (vapor) when it volatilizes after application. As a result, we noted in the preliminary assessment that there was a significant uncertainty about the relevance of an aerosol toxicity study to human exposures to volatilized chlorpyrifos. Although it is well accepted that bystander exposures will be to vapor rather than aerosol and, as such, that vapor is the most relevant form for evaluating potential bystander risks from chlorpyrifos volatilization, we lacked toxicity data based on vapor administration to test animals for chlorpyrifos and the chlorpyrifos-oxon at the time of the 2013 preliminary volatilization assessment.

Since the release of the preliminary volatilization assessment, Dow AgroSciences LLC conducted two high quality nose-only rat vapor phase inhalation studies for both chlorpyrifos and chlorpyrifos-oxon⁹ to address this uncertainty. These studies, as indicated in the attached memorandum *Chlorpyrifos: Reevaluation of the Potential Risks from Volatilization in Consideration of Chlorpyrifos Parent and Oxon Vapor Inhalation Toxicity Studies*¹⁰, have significantly altered our analysis of the hazards resulting from chlorpyrifos and chlorpyrifos-oxon vapor. Both studies evaluated the effects of vapor forms of chlorpyrifos and its oxon at 100% air saturation – in other words, the maximum amount that the air can hold and therefore the maximum possible exposure level. Even at the point of air saturation, however, these studies revealed that concentrations of the vapor forms are much lower than the levels seen in the earlier aerosol study. Indeed, no cholinesterase inhibition was observed in either volatility study. What is clear from these data is that the air cannot hold levels of volatilized chlorpyrifos or its oxon that are capable of causing adverse effects from cholinesterase inhibition – the end point of concern for chlorpyrifos. Accordingly, based on the new data, we no longer anticipate that there are human health risks of concern from exposure to the volatilization of either chlorpyrifos or chlorpyrifos-oxon. As such, EPA therefore intends to deny the volatilization component of your inhalation exposure claim when we publish our complete response to the petition in the Federal Register.

For efficiency purposes we do not intend to proceed with issuing a denial order of the six petition issues that we responded to in July 2012 and the volatilization portion of your inhalation exposure issue (the spray drift portion of your inhalation claim was granted) addressed today until after we complete our review of all remaining issues. Our understanding is that this approach is preferable to you as well. However, if you wish to begin the objections process for

⁸ EPA MRID 48139303: Acute Inhalation Exposure of Adult CrI:CD(SD) rates to particulate chlorpyrifos aerosols: Kinetics of Concentration-Dependent Cholinesterase (CHE) Inhibition in Red Blood Cells, Plasma, Brain and Lung; Authors: J. A. Hotchkiss, S. M. Krieger, K. A. Brzak, and D. L. Rick; Sponsor: Dow AgroSciences LLC.

⁹ W. Irwin. *Review of Parent Vapor Study Title. Date. U.S. EPA Office of Chemical Safety and Pollution Prevention. EPA. 6/25/14. D411959.*

W. Irwin. *Review of Oxon Vapor Study Title. Date. U.S. EPA Office of Chemical Safety and Pollution Prevention. EPA. 6/25/14. D415447.*

¹⁰ Also available at www.regulations.gov in docket EPA-HQ-OPP-2008-0850.

the denied claims and notify EPA in writing, we will publish a formal denial order for those claims, triggering your right to file objections under FFDCA section 408(g)(2).

Remaining Work

In our January 2013 letter¹¹ updating you on the status of our efforts to respond to your remaining petition issues, we indicated that we were pursuing a number of avenues that would eventually inform our response. One such effort was to solicit comments, which occurred late in the summer of 2012, from scientists within the federal government who have expertise in the areas of (1) the assessment of neurodevelopmental outcomes and (2) the use of magnetic resonance imaging. This peer review, *Federal Letter-Review of Chlorpyrifos Epidemiology Studies*¹², which contains a list of the Federal reviewers; the questions we asked the reviewers to consider during their review of the Rauh et al. study; our brief synopsis of Rauh et al. 2012 and our evaluation of the epidemiological aspects of the MRI study; the letters sent to each reviewer soliciting their input; and their review can be found in the chlorpyrifos registration review docket¹³. As we indicated previously, the results of the peer review will be considered when completing our RHHRA.

Coinciding with all of our work addressing your petition issues is our work on the RHHRA. I wanted to inform you of recent developments on that front. This past winter the EPA received a multi-route physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for chlorpyrifos and chlorpyrifos-oxon from Dow AgroSciences LLC. We have held several Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panels¹⁴ on the relevance and usefulness that a PBPK/PD model can provide in assessing a chemical's risks and two SAPs specifically on PBPK/PD and chlorpyrifos¹⁵. This PBPK/PD model was a decade in the making and can assess oral, dermal, and inhalation routes of exposure in both rats and humans. We have conducted a quality assurance (QA) assessment of the model. The QA assessment focused on ensuring that the model's structure and parameter values were accurately reflected and implemented in the computer code, as well as determining whether human data can be reasonably simulated by the model. Based upon our assessment this is a robust model. Instead of the use of standard defaults, the PBPK/PD model refines the extrapolation from animals to humans and across the human population. Additionally, the model will be useful for informing

¹¹ Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2007-1005-0097>.

¹² Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170>.

¹³ EPA-HQ-OPP-2008-0850 available at www.regulations.gov.

¹⁴ U.S. EPA, December 11 - 12, 2003: Physiologically-Based Pharmacokinetic/Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the N-Methyl Carbamate Pesticides: A Consultation, available at http://www.epa.gov/scipoly/sap/meetings/2003/121103_mtg.htm; U.S. EPA, December 2, 2004: Use of Pharmacokinetic Data to Refine Carbaryl Risk Estimates from Oral and Dermal Exposure, available at http://www.epa.gov/scipoly/sap/meetings/2004/120204_mtg.htm; U.S. EPA, December 3, 2004: The N-methyl Carbamate Cumulative Risk Assessment: Strategies and Methodologies for Exposure Assessment, available at http://www.epa.gov/scipoly/sap/meetings/2004/120304_mtg.htm; and U.S. EPA, August 16 - 17, 2007: Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides, available at http://www.epa.gov/scipoly/sap/meetings/2007/081607_mtg.htm.

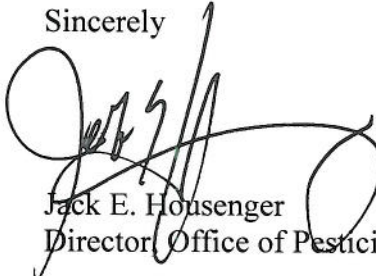
¹⁵ U.S. EPA, February 15-18, 2011: Chlorpyrifos Physiologically-Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) Modeling linked to the Cumulative and Aggregate Risk Evaluation System (CARES) available at <http://www.epa.gov/scipoly/sap/meetings/2011/021511meeting.html>.

our dose reconstruction work with respect to the epidemiological data, which is one of the items we have been working to address as it relates to your remaining petition issues.

In conjunction with our work on the RHHRA, we have worked over the last several months and continue to work with the technical and end use registrants to clarify application rates and uses that currently appear on labels. While this effort is designed primarily to ensure that labels reflect actual use, this effort should also help to preclude the overuse of chlorpyrifos in situations where the labeling may not clearly indicate application rates and uses on each crop. This effort will also help reduce uncertainties that could occur in the risk assessment resulting from unclear label language.

Finally, while we continue to make progress on your remaining three petition issues that deal with chlorpyrifos toxicity, for reasons noted above, we believe that a final response to your petition should correspond with completion of the RHHRA, which also addresses risks from human dietary exposure resulting from drinking water. We anticipate responding to your remaining three petition issues in connection with taking public comment on the RHHRA for chlorpyrifos by issuing either a proposed denial order or a proposed rule revoking tolerances, depending on the nature of the response. We anticipate releasing the RHHRA for public comment in December 2014 and would expect to issue a proposed denial order or a proposed revocation rule in the same time frame, accounting for any additional time that may be necessary to address procedural obligations that accompany Federal Register publication. As we have discussed with your counsel, the issuance of a proposed revocation rule constitutes a response to your petition. If the Agency determines that the petition should be denied following comments on the RHHRA and any proposed denial order, EPA would expect to publish a final denial order by the summer of 2015.

Sincerely

A handwritten signature in black ink, appearing to read 'Jack E. Housenger', written over a printed name and title.

Jack E. Housenger
Director, Office of Pesticide Programs



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: 6/25/14

SUBJECT: **Chlorpyrifos**: Reevaluation of the Potential Risks from Volatilization in Consideration of Chlorpyrifos Parent and Oxon Vapor Inhalation Toxicity Studies

PC Code: 059101

MRID No.: 49119501, 49210101

Petition No.: NA

Risk Assessment Type: Single Chemical;
Volatilization

TXR No.: NA

DP Barcode: D417105


Registration No.: NA

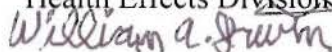
Regulatory Action: Registration Review


Case No.: NA

CAS No.: 2921-88-2

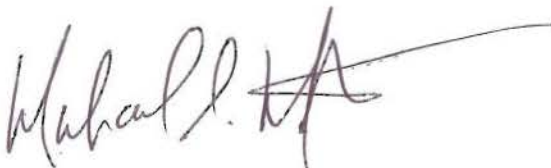
FROM:


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THROUGH: Michael Metzger, Branch Chief
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TO: Kevin Costello, Branch Chief
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Pesticide Re-Evaluation Division (PRD; 7508P)

The purpose of this memorandum is to reevaluate the potential human health risks from bystander exposure to the volatilization of chlorpyrifos and chlorpyrifos-oxon emitted from treated fields following the application of chlorpyrifos. The information and conclusions contained in this memo update and supersede those in the January 2013 Preliminary Evaluation

of the Potential Risks from Volatilization¹ assessment. Since the preliminary volatilization assessment was released for public comment in February 2013, new chlorpyrifos and chlorpyrifos-oxon inhalation toxicity data generated using saturated vapor phase administration were submitted to the Agency. The results of these studies have significantly changed how EPA considers the hazard to chlorpyrifos and chlorpyrifos oxon vapor. Based on the new data, there are no human health risks of concern anticipated for volatilization exposure to either chlorpyrifos or chlorpyrifos-oxon.

Volatilization Evaluation

Chlorpyrifos is currently undergoing registration review, EPA's periodic reevaluation of all registered pesticides. As part of registration review, the chlorpyrifos preliminary Human Health Risk Assessment (HHRA) was released for public comment in July 2011.² In the preliminary HHRA, potential risks to bystanders from spray drift and exposure from volatilization were identified as possible concerns. The potential risks from spray drift and the impact of potential risk reduction measures were assessed in a July 2012 memorandum³. Spray drift is the movement of aerosols and volatile components away from the treated area during the application process. To increase protection for children and other bystanders, chlorpyrifos technical registrants voluntarily agreed to lower application rates and other spray drift mitigation measures⁴. As of December 2012, spray drift mitigation measures and use restrictions appear on all chlorpyrifos agricultural product labels.

In January 2013, a preliminary assessment of the potential risks from volatilization was conducted. The assessment evaluated the potential risks to bystanders, or those who live and/or work in proximity to treated fields, from inhalation exposure to vapor phase chlorpyrifos and chlorpyrifos-oxon emitted from fields following application of chlorpyrifos. The results of the January 2013 assessment indicated that offsite concentrations of chlorpyrifos and chlorpyrifos-oxon may exceed the target concentration based on the toxicological endpoints used at that time. Specifically, for the January 2013 evaluation, points of departure (PoDs) were derived from an acute inhalation toxicity study⁵ using aerosolized chlorpyrifos which measured lung, plasma, RBC, and brain cholinesterase (ChE) inhibition; volatilization exposure was assessed only with use of the RBC and lung ChE data.

The January 2013 evaluation identified risks from off-field exposure but one significant area of uncertainty described in the preliminary assessment was the use of the aerosolized chlorpyrifos

¹ R. Bohaty, C. Peck, A. Lowit, W. Britton, N. Mallampalli, and A. Grube. Chlorpyrifos: Preliminary Evaluation of the Potential Risks from Volatilization. 1/31/2013. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399484, D400781.

² Available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0025>.

³ J. Dawson, W. Britton, R. Bohaty, N. Mallampalli, and A. Grube. Chlorpyrifos: Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. 7/13/12. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399483, D399485.

⁴ R. Keigwin. Spray Drift Mitigation Decision for Chlorpyrifos (059101). 7/2012. U.S. EPA Office of Chemical Safety and Pollution Prevention. EPA-HQ-OPP-2008-0850-0103.

⁵ EPA MRID# 48139303: Acute Inhalation Exposure of Adult CrI:CD(SD) Rates to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration-Dependent Cholinesterase (ChE) Inhibition in Red Blood Cells, Plasma, Brain and Lung; Authors: J. A. Hotchkiss, S. M. Krieger, K. A. Brzak, and D. L. Rick; Sponsor: Dow AgroSciences LLC.

inhalation toxicity study -- as opposed to chlorpyrifos vapor -- for evaluation of lung ChE resulting from field volatilization. Because field volatilization is the production and release of vapor into the atmosphere after sprays have settled on treated soils and plant canopies, it is well accepted that the vapor, rather than the aerosol, is the relevant form for evaluation of bystander volatilization exposures. However, EPA lacked chlorpyrifos vapor data at the time it conducted the preliminary volatilization assessment in 2013. Following the release of the preliminary volatilization assessment, Dow AgroSciences LLC conducted two, high quality nose-only vapor phase inhalation studies for both chlorpyrifos and chlorpyrifos-oxon⁶ to address this uncertainty. In both studies, female rats were administered a saturated vapor, meaning that the test subjects received the highest possible concentration of chlorpyrifos or chlorpyrifos-oxon which can saturate the air in a closed system. At these saturated concentrations, no statistically significant inhibition of ChE activity was measured in RBC, plasma, lung, or brain at any time after the six-hour exposure period in either study. Under actual field conditions, indications are that exposures to vapor phase chlorpyrifos and its oxon would be much lower as discussed in the January 2013 preliminary volatilization assessment. Summaries of the two vapor studies are included as an attachment to this document.

EPA's approach to human health risk assessment follows the four step paradigm established by the NAS (NRC, 1983). These steps include hazard identification, dose-response assessment, exposure assessment, and risk characterization, where risk is a function of both hazard and exposure. In the case of vapor exposure to chlorpyrifos or its oxon from volatilization, the new studies show no effects at saturation concentration in laboratory animals. In other words, no response related to toxicity to chlorpyrifos or its oxon were observed at the highest achievable concentration -- concentrations that likely far exceed those available to bystanders near treated fields. Accordingly, this finding leads to the conclusion that there is no hazard to chlorpyrifos or chlorpyrifos oxon vapor up to the highest possible concentration in the air. As such, if there is no hazard to the vapor for these pesticides, there is no risk. By extension, if there is no risk to chlorpyrifos or chlorpyrifos oxon, there is no need to perform quantitative risk estimates for off-field exposure. Although, in any risk assessment there is some uncertainty associated with extrapolating from laboratory animals to humans, the architecture of the lung is similar between rats and humans⁷ and, therefore, the Agency has confidence in this conclusion.

The preliminary volatilization assessment was released for public comment on February 2013 and closed May 2013. During this period comments were received regarding the exposure methodologies employed, and the uncertainties and assumptions in the preliminary volatilization assessment⁸. The Dow studies have made clear that volatilization of chlorpyrifos does not present a risk of ChE inhibition from inhalation of chlorpyrifos vapor, and none of the comments

⁶ W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Femal CD(SD): Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D411959. TXR# 0056694. EPA MRID# 49119501.

W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain, and Lung Cholinesterase Activity in Female CD(SD):Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D415447. TXR# 0056869. EPA MRID# 49210101.

⁷ W. Hofmann, L. Koblinger, T.B. Martonen. Structural Differences Between Human and Rat Lungs: Implications for Monte Carlo Modeling of Aerosol Deposition. Health Physics [1989, 57 Suppl. 1:41-6; Discussion 46-7].

⁸ All public comments and related documentation are available in docket EPA-HQ-OPP-2008-0850.

received included data or other information that would suggest that an appropriate vapor study does not represent the best means for assessing the potential for inhalation risk from volatilized chlorpyrifos. While a revised volatilization assessment is no longer needed, the EPA intends to review and respond to comments in a subsequent memorandum.

Attachment – Vapor Inhalation Study Summaries

Citation: Hotchkiss, JA, Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Femal CD(SD): CrI RATS (2013), MRID 49119501, Unpublished.

Sponsor: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

Summary:

In a special acute inhalation toxicity study (MRID 49119501), chlorpyrifos 97.6% a.i., (CPF, Lot # 7299412; TSN100759) was administered as a saturated vapor to 40 non-fasted CrI:CD Sprague Dawley strain female rats by nose-only inhalation exposure at a time-weighted concentration of 17.7 ppb (0.254 mg/m³) for 6 hours in comparison to control rats with 0 ppb. Rats were sacrificed immediately (0 hr) and at 2, 4, 6, or 12 hours after the end of exposure (n=8/exposure group/sacrifice time). Blood and tissues were isolated and processed to determine cholinesterase (ChE) activity in plasma, red blood cells (RBC), brain, and lung tissue. Whole blood samples from n=4 rats in each experimental group were analyzed to determine the concentration of chlorpyrifos (CPF), oxon and trichloropyrinol (TCP). An additional group of unexposed female control rats (n=8), which experienced the same laboratory and exposure tube acclimation and animal husbandry activities as the control and CPF exposure group animals, were sacrificed at the start of the air and CPF exposures. The oxon content in the chlorpyrifos was <0.1% and 1.4% in the test atmosphere.

No clinical effects indicative of cholinesterase inhibition were noted during the six-hour exposure period. In-life observations noted post-exposure were limited to soiling in four rats exposed to CPF and all rats appeared normal by test day 2. There were no decreases in ChE activity in any tissue which were significantly significant.

In the red blood cell samples, the ChE activity at all time points was comparable to control activity, with none having statistical significance. The ChE activities for the 17.7 ppb samples 0, 2, 4, 6 and 12 hours after exposure were 105.7%, 98.6%, 93.3%, 103.3%, and 110.5% of the 0 ppb control group, respectively.

In the plasma samples, the ChE activity for the 17.7 ppb samples 0, 2, 4, 6 and 12 hours after exposure were 94.9%, 106.3%, 85.8%, 105.4%, and 96.9% of the 0 ppb control group, respectively, with none having statistical significance. A slight decrease in ChE activity was measured at 4 hours post dose, however, it was not statistically significant and the trend was not consistent with the 2-hour and 6-hour results.

In the lung samples, the ChE activity for the 17.7 ppb samples 0, 2, 4, 6 and 12 hours after exposure were 88.6%, 94.8%, 88.8%, 93.8%, and 96.6% of the 0 ppb control group, respectively, with none having statistical significance. The 0 and 4-hour results had activity below 90%, however, this trend was not consistent with the 2, 6 and 12-hour findings.

In the brain samples, statistically significant ChE inhibition was not observed at any time point for the 17.7 ppb samples ChE activity at 0, 2, 4, 6 and 12 hours after exposure was 104.1%, 98.3%, 99.7%, 106.1%, and 101.7% of the 0 ppb control group. The 6-hour sample was the only time point with a statistically significant result, however, it was for an increase in ChE activity, not inhibition.

For chlorpyrifos, the blood levels ranged from the lower limit of quantitation (LLQ) to 0.335 pg/g during the time period after exposure. For TCP, the blood levels ranged from 12.4 to 90.4 pg/g during the time period after exposure. No oxon greater than or equal to the lower limit of quantitation (LLQ; 0.00015 nmole/g blood) was measured in the blood of any control (unexposed or 0 ppb-exposed) or CPF-exposed rat at any time point.

The purpose of the study was to determine pharmacokinetics and ChE inhibition of the RBC, plasma, brain and lung of rats exposed to a single dose of saturated vapor of chlorpyrifos. This purpose was achieved and no meaningful ChE inhibition was observed at the saturation concentration.

This study is classified as acceptable/non-guideline, however, since it was a special study, it does not fulfill any guideline requirement.

Citation: Hotchkiss, JA, Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain, and Lung Cholinesterase Activity in Female CD(SD):Crl Rats. MRID 49210101, Unpublished.

Sponsor: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

Summary:

In a special acute inhalation toxicity study (MRID 49210101), chlorpyrifos oxon (100% purity) was administered as a saturated vapor to 48 female CD(SD):Crl rats for six hours via nose-only exposure. This study was designed to assess the effect of an acute six-hour nose-only inhalation exposure to a saturated vapor concentration of chlorpyrifos oxon (oxon) on cholinesterase (ChE) activity in red blood cells (RBC), plasma, lung, and brain and to determine the blood concentrations of oxon and the metabolite 3,5,6-trichloro-2-pyridinol (TCP) in oxon-exposed rats. Female CD(SD):Crl rats were exposed six consecutive hours to filtered air (control) or a time-weighted average concentration of 35.3 $\mu\text{g}/\text{m}^3$ (2.58 ppb) oxon vapors using a flow-past nose-only inhalation exposure system. Rats were sacrificed immediately (0 hr) and 1, 2, 4, 8, or 24 hours after the end of exposure (n=8/exposure group/sacrifice time). Blood and tissues were isolated and processed to determine ChE activity in RBC, plasma, lung, and brain tissue. Oxon and TCP concentrations were determined in whole blood samples from four rats in each experimental group/sacrifice time.

During or after oxon exposure, no clinical signs of toxicity were noted in oxon-exposed rats at any time. There were no statistically significant decreases in ChE activity in any tissue

monitored. The only statistically significant changes in cholinesterase (ChE) activity were in the RBC and brain, however, increases of ChE activity occurred.

In the red blood cell samples, the ChE values for the 35.3 $\mu\text{g}/\text{m}^3$ samples at 0, 1, 2, 4, 8 and 24 hours after exposure were 93.6%, 110.6%, 113.9%, 116.5%, 102.2%, and 111.9%, respectively, of the 0 $\mu\text{g}/\text{m}^3$ control group, with none having both statistical significance and an activity decrease greater than 10%. The RBC ChE activity at 4 hours was statistically significant, however it was an increase in activity.

In the plasma samples, the ChE values for the 35.3 $\mu\text{g}/\text{m}^3$ samples at 0, 1, 2, 4, 8 and 24 hours after exposure were 103.0%, 107.5%, 101.5%, 136.7%, 101.0%, and 127.6%, respectively, of the 0 $\mu\text{g}/\text{m}^3$ control group, with none having statistical significance nor a decrease in ChE activity.

In the lung samples, the ChE values for the 35.3 $\mu\text{g}/\text{m}^3$ samples at 0, 1, 2, 4, 8 and 24 hours after exposure were 93.7%, 108.3%, 118.6%, 109.1%, 101.7% and 91.6%, respectively, of the 0 $\mu\text{g}/\text{m}^3$ control group, with none having statistical significance nor a decrease in activity greater than 10%.

In the brain samples, the ChE values for the 35.3 $\mu\text{g}/\text{m}^3$ samples at 0, 1, 2, 4, 8 and 24 hours after exposure were 100.5%, 106.2%, 102.0%, 100.9%, 101.2%, and 101.0%, respectively, of the 0 $\mu\text{g}/\text{m}^3$ control group, with none having both statistical significance and an activity decrease. The brain ChE activity at 1 hour was statistically significant, however it was an increase in activity.

At any time after exposure, no oxon was measured in the blood (lower-limit of quantitation; LLQ = 0.118 ng/g blood), however, blood TCP levels > LLQ (2.44 ng/g blood) were measured at the end of exposure and through eight hours post-exposure. No statistically significant inhibition of ChE activity by oxon was measured in RBC, plasma, lung, or brain at any time after exposure. The presence of TCP in the blood of oxon-exposed rats confirms that inhaled oxon vapor is absorbed by the respiratory tract, however, the oxon is rapidly metabolized to TCP and the oxon is not systemically bioavailable at blood levels \geq the LLQ of 0.118 ng/g blood (3.53×10^{-4} nmole/g blood). The six-hour No Observed Effect Concentration (NOEC) for inhaled oxon vapor is > 35 μg oxon/ m^3 air, based on the absence of statistically-significant cholinesterase inhibition in RBC, plasma, brain, or lung (the portal-of-entry tissue). The results of this study suggest that there is no cholinesterase-based hazard from inhalation of a saturated vapor concentration (35.3 $\mu\text{g}/\text{m}^3$) of chlorpyrifos oxon.

The purpose of the study was to determine pharmacokinetics and ChE inhibition of the RBC, plasma, brain and lung of rats exposed to a single dose of saturated vapor of chlorpyrifos. This purpose was achieved and no meaningful ChE inhibition was observed at the saturation concentration.

This study is classified as acceptable/non-guideline, however, since it was a special study, it does not fulfill any guideline requirement.