**APPENDIX 1-9. Degradate Line of Evidence**

*Identification of Transformation Products of Concern*:

Chlorpyrifos is known to form chlorpyrifos-oxon, 3,5,6-trichloro-2-pyridinol (TCP), and 3,5,6-trichloro-2-methoxypyridine (TMP). Chlorpyrifos may oxidize in the environment to form the biologically active compound, chlorpyrifos-oxon via oxidative desulfonation. Cleavage of the chlorpyrifos phosphorus ester bond yields the biologically inactive TCP. The phosphate moiety is required to bind within the active site of AChE. Available data also suggest that TCP may convert to TMP at relatively low concentrations (<10%) (**Figure B 1-9.1**).



**Figure B 1-9.1**. **Structures of chlorpyrifos, chlorpyrifos-oxon, TCP and TMP.** Red circles indicate the active moiety (the phosphate moiety is required to bind with the active site) of parent chlorpyrifos and chlorpyrifos-oxon.

Data are available for chlorpyrifos-oxon and three bird, one mammal, three fish, one amphibian, one aquatic invertebrate and one coral species (**Table B 1-9.1**). The available mortality, AChE, and reproduction endpoints for the parent and oxon are generally within an order of magnitude of each other, and indicate that chlorpyrifos-oxon is of similar or greater toxicity compared to the parent. Based on the available data, the greatest difference in toxicity is seen in aquatic-phase amphibians (tadpoles), where data indicate that chlorpyrifos-oxon is ~41 times more toxic than chlorpyrifos (again, see **Table B 1-9.1**). Therefore, chlorpyrifos-oxon is considered to be a transformation product of concern.

As discussed above, the primary degradate of chlorpyrifos is TCP. Toxicity data for TCP are available for three bird, one mammal, three fish, four aquatic invertebrate and three aquatic plant species (**Table B 1-9.2**). TCP has been shown to form at up to roughly one third of the applied parent and is more mobile and more persistent than chlorpyrifos. TMP is expected to have similar toxicity as TCP (due to their structural similarities) and is expected to form at lower concentrations in the environment than TMP. The potential exposure to TCP or TMP is not expected to contribute significantly to the overall risk because TCP and TMP are expected to be generally orders of magnitude less toxic than chlorpyrifos.

**Table B 1-9.1. Comparison of toxicity data available for chlorpyrifos and chlorpyrifos-oxon**

| **Test Species** | **Endpoint (units)** | **Chlorpyrifos Value (CI) (Ref.)** | **Chlorpyrifos-oxon Value (CI) (Ref.)** | **Comments** |
| --- | --- | --- | --- | --- |
| ***TERRESTRIAL*** | | | | |
| Northern bobwhite quail (*Colinus virginianus*) | LC50  (mg/kg-diet) | 283 (NA1) (MRID 44585401) | 175 (131 - 234)  (MRID 48602602) | Chlorpyrifos-oxon is ~1.6 times more toxic than chlorpyrifos (MRID 48602602 is supplemental because of poor stability of the oxon in the diet.  MRID 44585401 supplemental - temperature study; LC50 represents most sensitive endpoint at 27oC) |
| Mallard duck (*Anas platyrhynchos*) | LC50  (mg/kg-diet) | 136 (84-212) (MRID 00095007) | 523 (363 - 796)  (MRID 48365701) | Chlorpyrifos is ~3.8 times more toxic than chlorpyrifos-oxon |
| Northern bobwhite quail (*Colinus virginianus*) | LD50 (mg-kg-bw) | 32 (24-43) (MRID 41043901) | 8.8 (7.2 – 10.7)  (MRID 48365702) | Chlorpyrifos-oxon is ~3.6 times more toxic than chlorpyrifos |
| Passerine | LD50 (mg-kg-bw) | 10 (5.62 – 17.8) (MRID 40378401) (House sparrow, *Passer domesticus*) | >30 (NA) (MRID 48483703) (Zebra finch, *Taeniopygia guttata*) | Chlorpyrifos is >3 times more toxic than chlorpyrifos-oxon (MRID 48483703 is supplemental because of regurgitation) |
| Rat (*Rattus* sp.) | LD50 (mg/kg-bw) | 137 (NA) (MRID 00000179) | 794/870 (MRID 3F2884 4/1/91) | Chlorpyrifos is ~ 5.8 times more toxic than chlorpyrifos-oxon |
| Norway rat, adult - acute (*Rattus norvegicus*) | NOAEC/LOAEC (Plasma ChE) (mg/kg-bw) | 0.5/2 (NA) (48139301) | 0.1/0.5 (NA) (48139301) | Chlorpyrifos-oxon is ~ 4 times more toxic than chlorpyrifos (based on the LOAEC values) |
| Norway rat, adult - acute (*Rattus norvegicus*) | NOAEC/LOAEC (RBC ChE) (mg/kg-bw) | 0.5/2 (NA) (48139301) | 0.1/0.5 (NA) (48139301) | Chlorpyrifos-oxon is ~ 4 times more toxic than chlorpyrifos (based on the LOAEC values) |
| Norway rat, adult - acute (*Rattus norvegicus*) | NOAEC/LOAEC (Brain ChE) (mg/kg-bw) | 2/10 (NA) (48139301) | Not inhibited (48139301) | Chlorpyrifos is more toxic than chlorpyrifos-oxon |
| Norway rat, pup - acute (*Rattus norvegicus*) | NOAEC/LOAEC (Plasma ChE) (mg/kg-bw) | 0.5/2 (NA) (48139301) | 0.05/0.1 (NA) (48139301) | Chlorpyrifos-oxon is ~20 times more toxic than chlorpyrifos (based on the LOAEC values) |
| Norway rat, pup - acute (*Rattus norvegicus*) | NOAEC/LOAEC (RBC ChE) (mg/kg-bw) | 0.5/2 (NA) (48139301) | 0.1/0.5 (NA) (48139301) | Chlorpyrifos-oxon is 4 times more toxic than chlorpyrifos (based on the LOAEC values) |
| Norway rat, pup - acute (*Rattus norvegicus*) | NOAEC/LOAEC (Brain ChE) (mg/kg-bw) | 2/5 (NA) (48139301) | Not inhibited (48139301) | Chlorpyrifos is more toxic than chlorpyrifos-oxon |
| Norway rat, adult - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (Plasma ChE) (mg/kg-bw) | 0.1/0.5 (NA) (48139301) | 0.01/0.5 (NA) (48139301) | Similar toxicity (based on the LOAEC values) |
| Norway rat, adult - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (RBC ChE) (mg/kg-bw) | 0.1/0.5 (NA) (48139301) | 0.01/0.5 (NA) (48139301) | Similar toxicity (based on the LOAEC values) |
| Norway rat, adult - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (Brain ChE) (mg/kg-bw) | 0.5/1 (NA) (48139301) | Not inhibited (48139301) | Chlorpyrifos is more toxic than chlorpyrifos-oxon |
| Norway rat, pup - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (Plasma ChE) (mg/kg-bw) | 0.1/0.5 (NA) (48139301) | 0.01/0.5 (NA) (48139301) | Similar toxicity (based on the LOAEC values) |
| Norway rat, pup - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (RBC ChE) (mg/kg-bw) | 0.1/0.5 (NA) (48139301) | 0.01/0.5 (NA) (48139301) | Similar toxicity (based on the LOAEC values) |
| Norway rat, pup - chronic (*Rattus norvegicus*) | NOAEC/LOAEC (Brain ChE) (mg/kg-bw) | 0.5/1 (NA) (48139301) | Not inhibited (48139301) | Chlorpyrifos is more toxic than chlorpyrifos-oxon |
| ***AQUATIC*** | | | | |
| Bluegill sunfish (*Lepomis macrochirus*) | LC50 (µg a.i./L) | 1.7 (NA) (E006797) | 1.06 (0.57 – 1.4) (MRID 48483701) | Similar toxicity |
| Sheepshead minnow (*Cyprinodon variegatus*) | LC50 (µg a.i./L) | 136 (113 - 153) (15639) | 16 (13 - 21) (MRID 48756401) | Chlorpyrifos-oxon is 8.5 times more toxic than chlorpyrifos |
| Channel catfish (*Ictalurus punctatus*) | LOAEC (AChE) (µg a.i./L) | 13.4 (NA) (MRID 95013) | 7 (NA) (E67666) | Chlorpyrifos-oxon is ~2 times more toxic than chlorpyrifos |
| Yellow-legged frog (*Rana boylii*) | LC50 (µg a.i./L) | 204 (±544) (E118706) | <5 (NA) (E92498) | Chlorpyrifos-oxon is potentially > 41 times more toxic than chlorpyrifos |
| Daphnid (*Daphnia magna*) | EC50 (µg a.i./L) | 0.25 (NA) (E157799) | 2.78 (1.92 – 3.95) (48483702) | Chlorpyrifos is ~11 times more toxic than chlorpyrifos-oxon |
| Australian coral (*Acropora millipora*) | NOAEC/LOAEC (for larval metamorphosis) (µg a.i./L) | 0.3/1.0 (NA) (E100575) | 0.1/0.3 (NA) (E100575) | Chlorpyrifos-oxon is 3 times more toxic than chlorpyrifos |

1 NA = Not available.

**Table B 1-9.2. Comparison of toxicity data available for chlorpyrifos and TCP**

| **Test Species** | **Endpoint (units)** | **Chlorpyrifos Value (CI) (Ref.)** | TCP | **Comments** |
| --- | --- | --- | --- | --- |
| ***TERRESTRIAL*** | | | | |
| Northern bobwhite quail (*Colinus virginianus*) | LD50 (mg-kg-bw) | 32 (24-43) (MRID 41043901) | >2,000 (NA1) (MRID 418290-01) | Chlorpyrifos is > 62.5 times more toxic than TCP |
| Mallard duck (*Anas platyrhynchos*) | LC50  (mg/kg-diet) | 136 (84-212) (MRID 00095007) | >5,620 (NA)  (MRID 41829002) | Chlorpyrifos is > 41.3 times more toxic than TCP |
| Chicken (White leghorn cockerels, HY-Line 934E) | LD50 (mg a.i./kg-bw) | 34.8 (29.3 – 40.4) (MRID 00228759) | >1,000 (NA) (MRID 00228759) | Chlorpyrifos is > 29 times more toxic than TCP |
| Rat (*Rattus* sp.) | LD50 (mg/kg-bw) | 137 (MRID 00000179) | 794/870 (MRID 3F2884 4/1/91) | Chlorpyrifos is ~6 times more toxic than TCP |
| ***AQUATIC*** | | | | |
| Bluegill sunfish (*Lepomis macrochirus*) | LC50  (mg/a.i./L) | 0.0017 (NA) (E006797) | 12.5 (9.8 – 15.2) (MRID 41829003) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Rainbow trout (*Oncorhynchus mykiss*) | LC50  (mg/a.i./L) | 0.003 (NA) (MRID 95013) | 12.6 (7.6 – 20.8) (MRID 41829004) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Atlantic silverside (*Menidia menidia*) | LC50  (mg/a.i./L) | 0.0005 (0.0004 – 0.0007) (E11868) | 58.4 (44.5 – 76.7) (MRID 42245901) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Waterflea (*Daphnia magna*) | EC50 (mg a.i./L) | 0.25 (NA) (E157799) | 10.4 (4.9 – 17.9) (MRID 41829005) | Chlorpyrifos is ~ 42 times more toxic than TCP |
| Grass shrimp (*Palaemonetes pugio*) | LC50  (mg/a.i./L) | 0.00015 (0.00013 – 0.00018) (E92616) | 83.0 (71.4 – 97.0) (MRID 42245902) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Eastern oyster (*Crassostrea virginica*) | EC50  (shell deposition) (mg/a.i./L) | 0.032 (NA) (MRID 40228401) | 9.3 (3.6 – 24.2) (MRID 42245903) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Mosquito larvae (*Aedes aegypti*) | LD50 (mg a.i./L) | 0.0017 (0.0063 – 0.0082) (MRID 00228759) | 45.8 (40.2 – 51.7) (MRID 00228759) | Chlorpyrifos is orders of magnitude more toxic than TCP |
| Non-vascular aquatic plant | EC50 (cell density) (mg a.i./L) | 0.140 (NA) (*Isochysis galbana*) (MRID 40228401) | 2.9 (2.2 – 3.8) (*Selenastrum capricornutum)* (MRID 45312001) | Chlorpyrifos is ~ 14 times more toxic than TCP |
| 0.300 (0.270 – 0.340) (*Skeletonema costatum*) (MRID 40228401) | 2.0 (0.45 – 8.6) (*Anabaena flos-aquae*) (MRID 45312003) |
| 0.150 (0.120 – 0.180) (*Thalassiosira pseudonana*) (MRID 40228401) |
| Vascular aquatic plant | NOAEC/LOAEC (mg a.i./L) | 0.5/1.0 (NA) (growth)(*Lemna minor*) (E1555150) | 1.02/2.34 (NA) (frond number) (*Lemna gibba* G3) (MRID 45312002) | Chlorpyrifos is ~2 times more toxic than TCP |

1 NA = Not available.

**Mechanism of Formation of Oxon**

While exposure of organophosphate (OP) pesticides present in the environment is of concern for listed species, the corresponding oxon is also of concern. OPs that contain a phosphothionate group (P=S) may transform to the corresponding oxon analogue containing a phosphorus-oxygen double bond (P=O). While several studies have been conducted that indicate that OP and organothiophosphate chemicals that have sulfur double bonds to the central phosphorus atom generally form oxons during chemical disinfection by chlorine compounds (Magara*, et al*., 1994, Duirk and Collette, 2006; Wu and Laird, 2003), much less information is available on how the oxons form in the environment. The transformation occurs via oxidative desulfonation, which has been shown to occur through photolysis and may occur via other oxidative processes including soil metabolism.

Environmental fate studies submitted to EPA do not identify chlorpyrifos-oxon as a transformation product except in field volatility and air photolysis studies. In general, chlorpyrifos-oxon is expected to be less persistent than chlorpyrifos and may be present in air, soil, water, and sediment (see the Environmental Fate and Transport section of this document for more details).

**Ambient Monitoring Data**

Chlorpyrifos and chlorpyrifos-oxon have both been detected in various environmental media confirming environmental formation of chlorpyrifos-oxon. **Table** **B 1-9.3** provides a summary of ambient monitoring data including the maximum all time observed. The Environmental Fate and Transport section of this document provides additional ambient monitoring data details by program. Chlorpyrifos-oxon was also detected in a field volatility study. Nevertheless, these studies do not provide sufficient, consistent information on the levels of the oxon relative to chlorpyrifos, nor what conditions favor the oxon formation and/or persistence in the environment. As a result it is unclear the actual transport pathway of the oxon (*e.g.*, is it formed in the treated areas and transported via volatilization/runoff to waterbodies, does the parent compound volatilize and then transform to oxon in the atmosphere or at the receiving waterbody, or a combination), and how environmental estimated concentrations of the oxon should be modeled.

**Table B 1-9.3. Maximum observed concentrations of chlorpyrifos and chlorpyrifos-oxon in ambient monitoring data**

|  |  |  |
| --- | --- | --- |
| **Environmental Media** | **Chlorpyrifos** | **Chlorpyrifos-oxon** |
| Air | 1,340 ng/m3 | 230 ng/m3 |
| Surface Water | 14.7 µg/L | 0.054 µg/L |
| Sediment | 549 µg/kg | <3 µg/kg |
| Precipitation | No data | No data |

**Atmospheric Formation and Decay**

Long-range transport of chlorpyrifos may be caused by spray drift, volatilization, or particle transport. Chlorpyrifos has been shown to form chlorpyrifos-oxon via indirect and direct photolysis. Although chlorpyrifos-oxon may be slightly more persistent in air (*i.e.*, longer indirect photolysis half-life), chlorpyrifos-oxon is susceptible to indirect and direct photolysis.

EPISuite model AOPWin (atmospheric oxidation program) results indicate that the reaction rate for forming the oxon (reaction with S – sulfur, N – nitrogen, or hydroxy – OH) is only slightly faster than the rate of hydrogen abstraction. This suggests that the formation of the oxon is not quantitative. In addition, chlorpyrifos-oxon is subject to further degradation of the side chains. This is consistent with air photolysis data, where chlorpyrifos-oxon formed at maximum concentration of 50 ppb (or 11% of chlorpyrifos) approximately 5 hours after irradiation and direct and indirect half-life values less than one day. These results support the potential for near and long-range transport of both chlorpyrifos and chlorpyrifos-oxon.

**Potential Effects of Chlorpyrifos-oxon**

The primary exposure pathway of chlorpyrifos-oxon to non-target organism in the environment is thought to be a result of photolysis of chlorpyrifos in air following volatilization. The available air monitoring data indicate that, when detected, chlorpyrifos-oxon may be found at levels that are on the same order of magnitude as the parent. Additionally, the available toxicity data suggest that chlorpyrifos-oxon is of similar toxicity for most taxa when compared to the parent and is perhaps more toxic to some taxa (especially aquatic-phase amphibians) and less toxic to other (*e.g*., aquatic invertebrates). Therefore, in general, the effects of chlorpyrifos-oxon to exposed non-target organisms are likely to be similar to those of chlorpyrifos.

The likelihood of exposure to chlorpyrifos-oxon, however, may be less than the parent. This is because 1) chlorpyrifos-oxon is less persistent in the environment when compared to chlorpyrifos and 2) if oxon formation occurs in air, only a fraction of the applied chlorpyrifos would be transported in the air and subject to transformation (via reaction with hydroxyl radicals). The lower likelihood of exposure to chlorpyrifos-oxon appears to be supported by the available monitoring data where chlorpyrifos-oxon was detected less frequently than the parent (when considering samples where both were quantified).

For birds, mammals, reptiles, terrestrial-phase amphibians, and terrestrial invertebrates, potential exposure to chlorpyrifos-oxon is expected to be accounted for in the estimations of exposures for the parent based on the foliar half-life being used for modeling. The two available foliar dissipation half-lives for chlorpyrifos range from 2.9 to 4 days. The half-life of chlorpyrifos-oxon (<1 day) falls within this range. Given that the toxicity of chlorpyrifos and chlorpyrifos-oxon are similar for terrestrial animals (within an order of magnitude), any effects due to chlorpyrifos-oxon, would likely be captured by using a conservative half-life for chlorpyrifos.

For aquatic animals, chlorpyrifos-oxon is at most 41x more toxic than the parent based on the aquatic taxon that showed the greatest difference in toxicity using the available data (*i.e*., yellow-legged frog, E118706) (see **Table B 1-9.1**). Available aquatic monitoring data with chlorpyrifos-oxon and chlorpyrifos provide a maximum ratio of 0.054/14.7 µg a.i. (0.0037) (chlorpyrifos-oxon:chlorpyrifos) (see **Table B 1-9.3**). Therefore, although there is a potential for chlorpyrifos oxon to be more toxic to aquatic organisms than chlorpyrifos parent, available data indicate that the oxon is likely to be present at much lower concentrations than the parent. The ratio of the maximum expected oxon concentration (0.0037) and effects endpoint (41), both relative to chlorpyrifos, is 0.15 (*i.e*., 0.0037 x 41). Therefore, the risk of mortality associated with the oxon (exposure/effects) is expected to be low when compared to the risk (exposure/effects) associated with chlorpyrifos parent. Additionally, the small potential for increased risk of mortality associated with the oxon when compared to the parent is well within the margin of uncertainty associated with the mortality threshold for chlorpyrifos for fish and aquatic phase amphibians, which spans orders of magnitude[[1]](#footnote-1). Therefore, a lack of quantification of potential increases of mortality due to the potential presence of chlorpyrifos-oxon does not represent greater uncertainty compared to what is already inherent in the mortality thresholds for chlorpyrifos. For these reasons, in most cases, risks of mortality associated with the oxon are not expected to materially influence (*i.e*., change) any species-specific effects determination that is based on chlorpyrifos parent. Therefore, the potential risks associated with the oxon will not be quantitatively assessed. However, because of the uncertainties associated with the potential risks of the oxon (especially related to sub-lethal effects), the oxon will be qualitatively considered in the weight-of-evidence approach used for making effects determinations.

**References**

Duirk, S.E., and T.W. Collette. 2006. Degradation of chlorpyrifos in aqueous chlorine solutions: Pathways, kinetics, and modeling. *Environ. Sci. Technol*., 40(2): 546 – 551.

Glotfelty, D.E., Majewski, M.S. and J.N. Seiber. 1990. Distribution of several organophosphorus insecticides and their oxygen analogues in a foggy atmosphere. Environmental Science and Technology, 24 (3), 353-357.

Magara, Y., T. Aizawa, N. Matumoto, and F. Souna. 1994. Degradation of pesticides by chlorination during water purification. *Water Sci. Technol*., 30(7): 119 – 128.

Majewski, M.S. and P.D. Capel. 1995. Pesticides in the atmosphere: distribution, trends, and governing factors. Ann Arbor Press, Inc. Chelsea, MI.

Majewski, M.S., Zamora, C., Foreman, W.T., and C.R. Kratzer. 2006. Contribution of atmospheric deposition to pesticide loads in surface water runoff. United States Geological Survey. Open-file Report 2005-1307. Available online at: <http://pubs.usgs.gov/of/2005/1307/>.

Schomburg, C.J., Glotfelty, D.E., and J.N. Selber. 1991. Pesticide occurrence and distribution in fog collected near Monterey, California. Environmental Science and Technology, 25, 155 160.

Wu, J.G., and D.A. Laird. 2003. Abiotic transformation of chlorpyrifos to chlorpyrifos oxon in chlorinated water. *Environ. Toxicol. Chem*., 22(2): 261 – 264.

Zabik, J.M. and J.N. Seiber. 1993. Atmospheric transport of organophosphate pesticides from California's Central Valley to the Sierra Nevada Mountains. J environ qual. 22: 80-90.

1. For the HC05: SE = 1.13, CV = 0.79; for the threshold, the uncertainty bound due to slope values represents an additional order of magnitude. [↑](#footnote-ref-1)