**APPENDIX 1-9: Degradate line of evidence**

***Identification of degradates of concern***

Diazinon is known to form diazoxon and oxypyrimidine (**Figure B 1-9.1**). Diazinon may oxidize in the environment to form the biologically active compound, diazoxon. Cleavage of the phosphorus ester bond yields the biologically inactive oxypyrimidine. The phosphate moiety is required to bind within the active site of AChE. Available data also suggest that diazinon may convert to other degredates (GS-31144 and desethyl diazinon) at relatively low concentrations.



**Figure B 1-9.1. Structures of diazinon, diazoxon and oxypyrimidine.** Red circle indicates active moiety of parent and oxon degradate.

Data are available for two species of birds, one species of frog (aquatic phase) and two species of aquatic invertebrate exposed to diazoxon (**Table B 1-9.1**). The available mortality and reproduction endpoints for the parent and oxon are within an order of magnitude of each other (often with overlapping confidence intervals). AChE inhibition endpoints for one aquatic invertebrate indicate that the oxon is 3 orders of magnitude more toxic than the parent. This is expected since the toxicity of diazinon is attributed to transformation by organisms to the oxon. The available data indicate that diazoxon is of similar or greater toxicity compared to the parent. Therefore, diazoxon is considered to be a degradate of concern, whereas oxypyrimidine is not of toxicological concern.

For oxypyrimidine, data are available for two species of birds, one species of fish, one species of aquatic invertebrate and one species of algae (**Table B 1-9.2**). Oxypyrimidine is orders of magnitude less toxic compared to the parent. Therefore, oxypyrimidine is not of toxicological concern and will not be considered further.

**Table B 1-9.1. Comparison of toxicity data available for diazinon and diazoxon.**

| **Test species** | **Endpoint (units)** | **Diazinon value (CI)** | **Diazoxon value (CI)** | **Sources** | **Comments** |
| --- | --- | --- | --- | --- | --- |
| Northern bobwhite | LD50 (mg/kg-bw) | 5.2 (3.5-7.6) | 5.0 (4.1-6.3) | MRIDs 109015, 46579604, ECOTOX 37111and 37112 | Values show that diazoxon is of similar or slightly greater toxicity compared to diazinon. |
| 10 (7-13) |
| 13 (9-19) |
| 13 (8-21) |
| 14 (8-22) |
| 15 (10-24) |
| 15 (10-24) |
| 16 (11-24) |
| 16 (11-24) |
| 17 (11-25) |
| LC50 (mg/kg-diet) | 245 (178-234) | 72.3 (29-116) | ECOTOX 35243, MRID 46579602 | CIs do not overlap, suggesting that oxon is more toxic compared to diazinon  |
| NOEC (mg/kg-diet) | 35 (NA) | 4.53 (3.9-5.1) | ECOTOX 35482, MRID 48908401 | Data suggest that oxon is more toxic to reproduction. However uncertainty in comparison because diazinon study did not quantify residues (nominal values provided), test design is non-standard and dose spacing is different. In registrant study (MRID 41322902), NOEC = 32 (no LOEC). Endpoints observed at LOEC are different.  |
| LOEC (mg/kg-diet) | 50 (NA) | 17.9 (15.1-20.6) |
| Mallard duck | LC50 (mg/kg-diet) | 32 (16-64) | 104 (77-140) | MRIDs 40895302, 46579606, ECOTOX 35243 | Data suggest that diazinon and diazoxon are of similar toxicity. |
| 191 (138-253) |
| Yellow legged frog | LC50 (mg/L) | 7.5 (NA) | 0.76 (0.336 – 3.212) | ECOTOX 92498 | Lack of CI for diazinon confounds interpretation of relative sensitivities of parent and oxon. Upper bound of CI on oxon is only 2x lower than median value for parent. |
| Waterflea (Daphnia magna) | EC50 (uM) | 5,600 (NA) | 10,100 (NA) | ECOTOX 160445 | Endpoint is immobilization. Confidence intervals are not available. |
| Kuruma shrimp (*Panaeus japonicus*) | IC50 (uM) | 1194 (NA) | 1.27 (NA) | ECOTOX 3043 | Endpoint is AChE. Study tested different life stages. Average values presented here. Average ratio of endpoints for diazixnon to oxon was 942. Confidence intervals are not available. |

CI = confidence interval

NA = not available

**Table B 1-9.2. Comparison of available toxicity data for diazinon and oxypyrimidine.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test species** | **Endpoint (units)** | **Diazinon value (confidence interval)** | **Oxypyrimidine value**  | **Sources** | **Comments** |
| Bobwhite quail | LD50 (mg/kg-bw) | 5.2 (3.5-7.6) | >2060  | MRIDs 109015, 46579605, ECOTOX 37111and 37112 | No mortalities were observed in oxypyrimidine test. Values show that oxypyrimidine is orders of magnitude less toxic compared to diazinon. |
| 10 (7-13) |
| 13 (9-19) |
| 13 (8-21) |
| 14 (8-22) |
| 15 (10-24) |
| 15 (10-24) |
| 16 (11-24) |
| 16 (11-24) |
| 17 (11-25) |
| LC50 (mg/kg-diet) | 245 (178-234) | >4910  | ECOTOX 35243, MRID 46579603 | Only one mortality observed at 4910 mg/kg-diet oxypyrimidine (not statistically significant). Values show that oxypyrimidine is orders of magnitude less toxic compared to diazinon. |
| Mallard duck | LC50 (mg/kg-diet) | 32 (16-64) | >4990  | MRIDs 40895302, 46593301, ECOTOX 35243 | No mortalities were observed in oxypyrimidine test. Values show that oxypyrimidine is orders of magnitude less toxic compared to diazinon. |
| 191 (138-253) |
| Rainbow trout | LC50 (mg/L) | 0.09 | >101 |  | Values show that oxypyrimidine is orders of magnitude less toxic compared to diazinon. |
| Waterflea | EC50 (mg/L) | 0.00021 | >102 |  |
| Green algae | EC50 (mg/L) | 3.7 | >109 |  |

**Mechanism of Oxon Formation**

The chemical transformation process of OPs involves the substitution of the sulfur atom in the P–S bond of the organophosphate pesticide with an oxygen atom. While several studies have been conducted that indicate that OP and organodithiophosphate 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 natural environment. The transformation occurs via oxidative desulfonation, which could potentially occur through photolysis and aerobic metabolism, as well as other oxidative processes (*e.g.,* reaction with hydroxyl radicals and ozone).

A number of studies have documented atmospheric transport and deposition of pesticides apparently from the Central Valley to the Sierra Nevada Mountains (Fellers *et al*., 2004, Sparling *et al*., 2001, LeNoir *et al*., 1999, and McConnell et al., 1998). Prevailing winds blow across the Central Valley eastward to the Sierra Nevada Mountains, transporting airborne industrial and agricultural pollutants into Sierra Nevada ecosystems (Fellers *et al.*, 2004, LeNoir *et al*., 1999, and McConnell *et al*., 1998). Available literature has also documented oxon detections in air, rain, fog (Majewski and Capel, 1995) and surface waters in the United States (USGS, 2011). Although these studies provide evidence that the oxon is present in the environment, these studies do not provide sufficient, consistent information on the levels of the oxon degradate relative to the parent, nor what conditions favor the oxon formation and/or persistence in the environment. As a result the actual transport pathway(s) of the oxon are unclear (*e.g.*, is it formed in the treated areas and transported via volatilization/runoff to waterbodies, or does the parent compound volatilize and then transform to oxon in the atmosphere or at the receiving waterbody) and how environmental estimated concentrations of the oxon could best be modeled.

Diazoxon was not detected in registrant-submitted environmental fate studies in which it was monitored except for one aerobic soil metabolism study, where it was observed at a maximum amount of 0.6% applied radioactivity and in an atmospheric degradation study. In many of the studies the registrants did not indicate that they had analyzed for the oxon and in cases where the oxon was evaluated the limits of quantitation for diazoxon were high (10 to 20 µg/kg-soil). Additionally, there were unidentified residues (maximums percent radioactivity associated with unidentified residues ranged from 0.9 to 13%) in the environmental fate studies that could have been associated with diazoxon. In an air photolysis study (MRID 49049901), diazoxon was present in air before exposure to light at 9.60 parts per billion. In order to generate diazinon in the air, the compound was heated and the study author hypothesized that diazoxon formed with the heating of diazinon and was not due to photolysis. Diazoxon is not persistent in air, and has a measured atmospheric degradation half-life (estimated for the average 12-hour day time concentration of hydroxyl radicals at 30oC) of 4.1 hours (MRID 49049902). The only submitted environmental fate study for diazoxon is an air photolysis study which estimated a half-life of 4 hours in air.

**Estimated** **Atmospheric Formation and Decay**

OP insecticides may be converted to their oxons or other products by reaction with oxidants such as hydroxyl radical, ozone, or nitrate radical in the atmosphere. The oxons themselves may also be degraded by the same oxidants, which will limit their persistence in the atmosphere, and thus their potential for transport to distant locations.

We used the EPISuite model AOPWin (atmospheric oxidation program) to estimate reaction rate constants of diazinon with these oxidants. **Table B 1-9.3** lists the estimated rate constants (all in E-12 cm3/molecule-sec) for various reaction of hydroxy radical, and the estimated atmospheric half-life, assuming an atmospheric concentration of 1.5E+6 molecules of OH radical per cm3, and a 12-hour day. AOPWin did not predict any reaction with ozone or nitrate radical.

**Table B 1-9.3. Reaction Rate Constants and half-lives for diazinon Reacting with Hydroxyl Radical**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Chemical | Hydrogen Abstraction | Reaction with S, N, or OH | Addition to Triple Bond | Addition to Aromatic Ring | Addition to Fused Ring | Half-life of OH Reaction (Hours) |
| Diazinon | 41.04 | 53 | 0 | 2.6404 | 0 | 1.328 |

The data in **Table B 1-9.3** indicate that the reaction forming the oxon (reaction with S – sulfur, N – nitrogen, or hydroxy – OH) is the fastest reaction predicted with a rate constant of 53. However, the competing reaction of hydrogen abstraction, which would attack and degrade the alkyl side chains of the phosphate ester, are almost as fast (rate constants 24 to 41). This means that the formation of oxons would not be 100% of the parent OP reacting, and that any oxons formed would be subject to further degradation of the side chains. Both processes are reflected in the overall half-life of the parent OP in **Table B9-3**. Reactions of hydroxyl radical with aromatic rings are much slower (rate constants 0.04 to 2.60) and would not result in much product.

Degradation of parent OPs to less than 1% of the starting concentration would occur with seven half-lives, or 9.3 hours for diazinon. Maximum production of oxons would occur within this timeframe. Any oxons formed would be subject to further degradation by hydroxyl radical by the same reaction mechanisms, except for reaction with sulfur (S) which would already be complete. The half-life of diazoxon with the remaining processes, hydrogen abstraction and addition to aromatic ring, is 2.9 hours. The time to degradation to less than 1% of the maximum amount of oxon formed would thus be 20.6 hours (diazoxon).

**Ambient Monitoring Data**

Ambient monitoring data are available for the oxon to indicate the presence of the oxon in air and water. In general, diazinon was more frequently detected compared to diazoxon. When the two were detected together, they were generally at concentrations that were of the same order of magnitude. These data are limited in that there is no direct link between a pesticide application and the presence of the oxon. In addition, it is unclear how the oxon arrived in the media.

*Water*

The detection frequency of diazoxon in surface water is lower (0.6 to 8%) than that for parent diazinon (see detailed discussion of diazinon monitoring data in the environmental fate characterization). In surface water monitoring data wherein residues of both diazinon and diazoxon were detected, the ratios of the concentrations of diazoxon to diazinon ranged from 0 to 0.5. Diazinon and diazoxon were sometimes detected in the same samples and sometimes did not co-occur in samples.

The USGS analyzed a total of 1,499 samples across sites throughout the United States for diazoxon between 2002 and 2014 (USGS, 2015). Detections occurred in 2004 in California and Texas. Concentrations ranged from not detected to 0.06 µg/L with the highest detected being detected in 2004. The limit of quantitation ranged from 0.006 to 0.045 µg/L based on the range of ‘less than’ values reported in the dataset.

STORET/Water Quality Exchange (WQX) is a repository for water quality, biological, and physical data maintained by the USEPA. Data are submitted by states, tribes, and others. Surface water samples were collected and analyzed for diazoxon. In 2009 in Washington, 10 samples were collected and diazoxon all samples were below the quantitation level. Diazoxon was not detected in 1,659 samples collected in Minnesota between 2012 and 2013.

In the CDPR database, 773 samples were analyzed to determine whether they contained diazoxon between 1991 and 1995. Diazoxon was detected in five samples at 0.06, 0.08, 0.21, 0.39, and 0.43 µg/L. The limit of quantitation ranged from 0.05 to 0.1 µg/L. Detections occurred in Merced and San Joaquin counties in Spillways, wasteways, and a slough. These data are limited in that the study was conducted at a time when diazinon use was permitted at much higher levels than allowed today and on many other use sites.

*Air*

Zabik and Seiber (1993) collected air samples in 1990 and 1991 from a national park in the Sierra Nevada Mountains. Diazinon was detected at concentrations ranging <1.4 (LOQ)-10,000 pg/m3. Diazoxon concentrations ranged 4-3,000 pg/m3. The authors reported that 26% of the parent was converted to the oxon. In paired samples, the ratio of the oxon to the parent ranged 0.068-3.9 (N = 34).

*Rain*

In a study of diazinon and diazoxon concentrations in precipitation in California, diazoxon was measured in 39% of samples (n=137), with mean and maximum concentrations of 0.041 and 0.300 µg/L, respectively. Diazinon was detected more frequently in this study (93%) with mean (0.149) and maximum (2.2) concentrations that were comparable to those of the oxon (Majewski et al. 2006).

Zabik and Seiber (1993) also collected rain samples deposited in the Sierra Nevada Mountains. In their limited samples, they detected diazoxon at lower levels compared to the parent.

*Fog*

Diazinon and diazoxon have been quantified fog in two different studies conducted in California. In 1986, diazinon concentrations ranged 0.31-18 ug/L and diazoxon concentrations ranged 0.42-28 ug/L (n = 6). The ratios of the oxon to the parent ranged 0.056-7.1, where the majority of the samples had concentrations of the two that were on the same order of magnitude (Glotfelty *et al.* 1990). In 1987, diazinon concentrations ranged 0.15-4.8 ug/L and diazoxon concentrations ranged 1.9-11 ug/L (n = 5). The ratios of the oxon to the parent ranged 0.067-13, where the majority of the samples had concentrations of the two that were on the same order of magnitude (Schomburg *et al.* 1991).

**Potential Effects of Diazoxon**

Available monitoring data indicate that, when detected, diazoxon is generally detected at lower concentrations compared to the parent. In air, detections of the parent and oxon are similar in magnitude. Given that diazoxon is of similar or somewhat greater toxicity compared to the parent, the effects of diazoxon on mortality and reproduction to exposed non-target organisms are likely to be similar to those of the parent.

The likelihood of exposure to diazoxon may be less than that of the parent. This is because 1) diazinon is less persistent in the environment when compared to diazinon and 2) if oxon formation occurs in air, only a fraction of the applied diazinon would be transported to the air and subject to transformation. Lower exposure to diazoxon is suggested by the available monitoring data in which diazoxon was detected less frequently than the parent (when considering samples where both chemicals were quantified).

For birds, the uncertainty associated with not quantifying diazoxon may be less because of the conservative nature of the foliar dissipation half-life that is used for diazinon. Available half-lives range over an order of magnitude, i.e., 0.4-5.3 days. The dissipation of diazinon is conservatively represented by a 90th percentile value of 5.2 days. The half-life of diazoxon in air (4 hours) falls within this range. Given that the toxicity of diazinon and diazoxon are almost equal for birds (endpoints are within a factor of 3), any effects due to diazoxon, would potentially be captured by using a conservative half-life for diazinon and the toxicity endpoints based on exposure to diazinon.

The most conservative assumption related to mortality to aquatic organisms would be that diazoxon is 10x greater than the parent (based on the yellow-legged frog data from ECOTOX 92498). Available aquatic monitoring data with diazoxon and diazinon provide a maximum ratio of ½. When taken together, this suggests that when both diazinon and diazoxon occur in water, the mixture’s effect could exert as much as a 5-fold greater effect. This amount of uncertainty is less significant compared to the uncertainty already incorporated into the mortality threshold for diazinon 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 presence of diazoxon does not represent greater uncertainty compared to what is already inherent in the mortality thresholds for diazinon.

Diazoxon exposures may be most of concern for potential increases in AChE inhibition in aquatic invertebrates. Available data for one test species of shrimp indicated that diazoxon resulted in almost a 1000x greater effect (IC50) compared to the parent alone (ECOTOX 3043). This would indicate that in cases where diazoxon is present, there could be a concern for AChE inhibition in aquatic invertebrates at levels where there is no concern based on the parent alone.

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1. for the HC05: SE = 115, CV = 0.48; for the threshold, the uncertainty bound due to slope values represents an additional order of magnitude [↑](#footnote-ref-1)