Appendices to

Risks of Diazinon Use to the Federally Listed California Red-Legged Frog

(*Rana aurora draytonii*)

Pesticide Effects Determination

Appendix A. ECOTOX Open Literature Reviews

A total of 2,335 references were identified for diazinon in a search of ECOTOX conducted in September 2006 Of these, approximately 27 studies contained toxicity endpoints that were more sensitive than those listed in the 2002 IRED. Reprints for each of these studies were reviewed to determine whether the studies could be used either quantitatively or qualitatively to describe the potential effects of diazinon on aquatic organisms. Below is a brief description of each of the studies along with any uncertainties that were identified during the review. The bolded number preceding each of the citations represents the ECOTOX reference number.

ECOTOX Record Number and Citation: 18129 Werner, I. and R. Nagel. 1997. Stress Proteins HSP60 and HSP70 in three Species of Amphipods Exposed to Cadmium, Diazinon, Dieldrin and Fluoranthene. Environmental Toxicology and Chemistry. 16(11): 2393 – 2403.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Article reports 24-hr LC_{50} value determined as part of a range finding test for measuring response of heat shock proteins. Diazinon concentrations determined using immunoassay (EnviroGard test kit; Millipore, Bedford, MA). Three replicate test containers each containing 150 mL. Control and solvent controls run; no solvent used for diazinon. Ten test species (freshwater *Hyalella azteca* and the marine *Rhepoxynius abronius*); 20 estuarine *Ampelisca abdita* because of smaller size. Filtered (0.22 µm) dilution water obtained from Bodega and San Francisco bays for saltwater and freswater studies. Dissolved oxygen 6.9 – 9.0 mg/L; pH ranged from 7.7 to 8.4.

	24-hr	48-hr
H. azteca	30 µg/L	19 µg/L
A abdita	21 µg/L	10 µg/L
R. abronius	9.2 µg/L	--

Remainder of study examines heat shock protein responses; the relevancy of these data to assessment endpoints is not determined quantitatively.

Description of Use in Document (QUAL, QUAN, INV): Qualitative

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ECOTOX Record Number and Citation: 15687 Sancho, E., M. D. Ferrando, M. Gamon and E. Andreu-Moliner. 1994. Uptake and Clearance of Diazinon in Different Tissues of the European Eel (*Anguilla anguilla* L.) Biomedical and Environmental Sciences 7: 41 – 49.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Study is deemed to be of low utility:

Wild-caught eels
Test animals did not respond to food and therefore may have been fasted for 2 weeks before the study and during the 96-hr study.
Tap water is used.
No mention is made whether concentrations are measured therefore, the concentrations are presumed to be nominal; the accumulation study did measure concentrations though.
Aquaria are aerated.

	24-hr	48-hr	72-hr	96-hr
A. anguilla	164 µg/L	114 µg/L	92 µg/L	85 µg/L

Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: Eels are not the most sensitive speices tested with diazinon. The study provides useful information for qualitative species sensitivity distribution.

Limitations of Study: The fact that the test animals were essentially fasted for at least 2 weeks prior to test initiation raises serious concerns regarding the utility of these data. Extensive fasting would likely mobilize the animal's fat reserves. Given the uncertain chemical exposure history for the eels, it is uncertain what effect the fasting may have on the study's ability to detect treatment effects.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 1055 Ferrando, M. D., E. Sancho, and E. Andreu-Moliner. 1991. Comparative Acute Toxicities of Selected Pesticides to *Anguilla anguilla*. Journal of Environmental Science and Health B26: 491 – 498.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Wild-caught eels (Albufera Lake, Valencia, Spain)

Acclimatized for 2 weeks; however, animals did not respond to feeding attempts.
Glass aquaria (40 L) containing 35 L test solution; 4 replicates with10 fish per replicate per treatment. (Diazinon 92% a.i.) Controls run. No mention of whether concentrations were measured.

	24-hr	48-hr	72-hr	96-hr
A. anguilla	160 µg/L	110 µg/L	90µg/L	80µg/L

The results of this study are strikingly similar to results reported in the 1994 publication by Sancho. It is unclear whether this is the same study.

Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: Eels are not the most sensitive speices tested with diazinon. The study provides useful information for qualitative species sensitivity distribution.

Limitations of Study: The fact that the test animals were essentially fasted for at least 2 weeks prior to test initiation raises serious concerns regarding the utility of these data. Extensive fasting would likely mobilize the animal's fat reserves. Given the uncertain chemical exposure history for the eels, it is uncertain what effect the fasting may have on the study's ability to detect treatment effects.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist
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ECOTOX Record Number and Citation: 16043 Norberg-King, T. J. 1987. Toxicity Data on Diazinon, Aniline and 2, 4-Dimethylphenol. Memo to Charles Stephan, ERL Duluth from the U.S. EPA Environmental Research Laboratory in Duluth.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Summary of diazinon (85% ai) acute (48-hr) toxicity tests with *Ceriodaphnia dubia* (in-house culture; <24 hrs old) using water from various sources: Lake Superior water (LSW), reconstituted water (RCW), diluted mineral artificial water (DMW) and Lake Superior culture water (water enriched by previous goldfish use). Daphnia in most of the studies were fed using green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and yeast concentrate. Test volumes of 12.5 ml in replicate with two replicates per test concentration. Diazinon dissolved in methanol

	48-hr
DMV	0.57 µg/L
LSW	0.66 µg/L
RCW	0.57 µg/L
LSCW	>1.0 µg/L

Limitations of Study: Concentration of methanol is not reported. It is unclear whether the control is a solvent control or neat control. Some studies had concentrations measured in the treatment units while others measured diazinon in the stock solutions.

A 7-day chronic toxicity study is also reported using one daphnid (<6-hr old) in 15 ml of test solution (DMW) with 10 reps per treatment concentration; solutions renewed daily and all concentrations were measured.

NOEC = 0.22 µg/L; LOEC = 0.34 µg/L (mean number of young/female).

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freshwater nonvascular aquatic plants to diazinon.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 16547 Oh, H. S., S. K. Lee, Y. H. Kim and J. K. Roh. 1991. Mechanism of Selective Toxicity of Diazinon to Killifish (*Oryzias latipes*) and Loach (*Misgurnus anguillicaudatus*). Aquatic Toxicology and Risk Assessment: Fourteenth Volume, ASTM STP 124. M. A. Mayes and M G. Barron (editors), American Society for Testing and Materials. Pp 343 – 353.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Study reports a 96-hr LC_{50} value for killifish (LC_{50}= 3,910 µg/L) and loach (LC_{50}=270 µg/L); however, the methods section does not indicate that any such test was undertaken.

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of fish to diazinon.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 821 Ankley, G. T., J. R. Dierkes, D. A. Jensen, and G. S. Peterson. 1991. Piperonyl Butoxide as a Tool in Aquatic Toxicologicl Research with Organophosphate Insecticides. Ecotoxicology and Environmental Safety 21 (3): 266 – 274.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: *Ceriodaphnia dubia*, *Daphnia magna* and *Daphnia pulex* obtained from in-house cultures; all test organisms <u><</u>48 hrs old. Five organisms per test replicate, two replicates per treatment with 10 mL per treatment container. Tests conducted at 25oC; control used 10% mineral water (Perrier, Vergeze, France) diluted in high purity water from a Millipore system.

	48-hr LC_{50}
C. dubia	0.50 µg/L
D. magna	0.80 µg/L
D. pulex	0.65 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

Limitations of Study: Specific purity of diazinon is not provided; report simply cites purities ranging from 95 to 99%. Test concentrations are nominal. Methanol is used as a co-solvent; report states that concentration did not exceed 1.5% and this is "well below" the 48-hr LC_{50} for methanol. However, no solvent control is run and it is unclear why the control contained 10% mineral water.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 4009 Fernández-Caladerrey, A., M. D. Ferrando and E. Andreu-Moliner. 1994. Effect of Sublethal Concentrations of Pesticides on the Feeding Behavior of *Daphnia magna*. Ecotoxicology and Environmental Safety 27: 82 – 89.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: *Daphnia magna* from the Laboratory for Biological Research in Aquatic Pollution (Gent, Belgium) and cultured in laboratory. Diazinon 92% ai was dissolved in acetone. Study procedure according to EEC standard. Six concentrations plus a control acetone (0.06 mg/L) consisting of 3 replicates with 10 neonates (<24 hr old) placed in 30 ml glass beaker containing 25 ml test solution. Animals were fasted and study was conducted under static conditions.

	24-hr LC_{50}
D. magna	0.9 µg/L diazinon
	0.62 mg/L endosulfan

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon and endosulfan.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist
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ECOTOX Record Number and Citation: 5311 Dennis, W. H., A. B. Rosencrance and W. F. Randall. 1980. Acid Hydrolysis of Military Standard Formulations of Diazinon. Journal of Environmental Science Health, Part B. Pestic Food Contam. Acric. Wastes, B15(1): 47 – 60.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Young-of-the-year bluegill sunfish (*Lepomis macrochirus*; 0.8 g) from an unspecified source were exposed to diazinon (88.1% ai) for 96 hrs in a static system. Five-gallon glass jars containing 15 L treatment solution and contained 10 fish per rep and three reps per treatment. Mortality and treatment concentrations were measured every 24 hours. Well water used in study with alkalinity of 138 mg/L as $CaCO_3$.; temperature 20 $\pm$ 1oC

	96-hr LC_{50}
Bluegill	120 µg a.i./L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freswater fish to diazinon.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

Limitations of Study: In this study technical grade diazinon is more toxic than the formulated products tested (Diazinon EC; LC50 530 µg a.i/L

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ECOTOX Record Number and Citation: 885 Sanders, H. O. 1969. Toxicity of Pesticides to the Crustacean *Gammarus lacustris*. Technical Papers of the Bureau of Sport Fisheries and Wildlife. U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington DC.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Laboratory stock cultured from scuds (Gammarus lacustris) collected at pond near the Fish-Pesticide Research Laboratory (Denver, CO). Reconstituted water (pH = 7.1; alkalinity = 30 ppm). Glass aquariums (5.7 L) containing 4 L of tests water. Ten 2-month old scuds placed in each aquarium; then 2 hours later, test material was added to aquaria. Test conducted at 21oC (70oF) Appears that only neat control and not a solvent (ethanol) control was run. Procedure indicates that emulsifiable concentrates and wettable powders were dissolved in deionized water while technical grade pesticides were dissolved in ethanol; however the article does not discuss what form the diazinon was in. Ethanol concentration never exceeded 1 mL per liter; however, 1 ml/l is a very high concentration of co-solvent. The endpoints reported in the study are no more sensitive than what is already reported for aquatic invertebrates..

	24-hr	48-hr	96-hr
Scud	800 µg/L	500 µg/L	200 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 18190 Bailey, H. C., J. L. Miller, M. J. Miller, L. C. Wiborg, L. Deanovic and T. Shed. 1997. Joint Acute Toxicity of Diazinon and Chlorpyrifos to *Ceriodaphnia dubia*. Journal of Environmental Toxicology and Chemistry. 16(11): 2304-2308.
Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Diazinon (99% ai) dissolved in 100% methanol. Dilution water obtained from everse osmosis-treated well water brought to moderately hard standard. Nominal test concentrations of 0.05, 0.10, 0.20, 0.40 and 0.80 µg/L. *Ceriodaphnia dubia* (<24 hr old) obtained from in-house laboratory culture. Exposures conducted in 20-l glass scintillation vials containing 18 ml of solution. Four replicates containing five neonates in each used at each of the five test concentrations; studies were static tests as 25 + 1oC with a 16 hr day and 8 hr night photoperiod. Initial concentrations of diazinon determined through ELISA. Animals fasted through study period.

	24-hr	48-hr	72-hr	96-hr
Ceriodaphnia	0.75 µg/L	0.48 µg/L	0.40 µg/L	0.35 µg/L
	0.58 µg/L	0.58 µg/L	0.35 µg/L	0.32 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of fish to diazinon.

Limitations of Study: This study has a relatively good methodology; however, diazinon was dissolved in methanol and the final concentration of methanol is not reported. Also, a solvent control is not reported.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 19300 Harris, M. L., C. A. Bishop, J. Struger, B. Ripley and J. P. Bogart. 1998. The Functional Integrity of Northern Leopard Frog (*Rana pipiens*) and Green Frog (*Rana clamitans*) Populations in Orchard Wetland. II. Effects of Pesticides and Eutrophic Conditions on Early Life Stage Development. Environmental Toxicology and Chemistry 17(7): 1351 – 1363.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Leopard frog adults obtained from R. Elinson (Hazen Frog Farms, Alburg, VT) and from wild-caught adults. Green frog adults were wild-caught. Animals were induced with 0.1 µg luteninzing hormone-releasing hormone or with whole frog or toad pituitary extracts.

Laboratory assays conducted in 250-ml beakers maintained at 19.5 $\pm$ 1.5oC for leopard frogs and 19.5 $\pm$ 0.6 and 18.6 $\pm$ 0.6oC for green frog assays. Photoperiod of 12:12 hr light:dark maintained. Beakers contained 10 individuals with 2 or 3 replicates per treatment. Tests initiatated at 9 hours post-fertilization (Gosner developmental stage 8/9). Larvae fed boilded lettuce (0.5 g) every other day; rations were increased to 1 g after approximately 1 week. Tests continued for 2 weeks (1993) for both species and for 3 weeks (1994) with green frogs. At test temination, survival, hatching success and tadpole growth rates determined.

Green frogs (Gosner stage 8 embryos through stage 25 tadpoles) were also continuously exposed for 13-day static renewal (4 day) toxicity tests to Basudin® 500 EC and technical grade diazinon. After 4 days, treatment solutions were replaced with reference pond water and embryos hatched and began feeding in "uncontaminated" conditions. After 7.5 day in reference water (with renewal every second day) treatment solutions were reintroducted. Treatment concentrations of Basudin® 500EC were 0.001, 0.01, 0.1, 1.0, 10 and 25 µg/L; treatment concentrations for technical grade diazinon were: 0.5, 5 and 50 µg/L. Results presented below are for technical grade diazinon; formulated end-product appears to be less toxic than the technical grade.

	96-hr LC_{50}	16-day LC_{50}
Green Frog	>50 µg/L	5 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of aquatic-phase amphibians to diazinon.

Limitations of Study: Laboratory studies appeared to be conducted using reference pond water; however, background pesticide residues were not analyzed at the time of the study. It is also unclear whether controls were run. The 16-day study was with feeding.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number and Citation: 3664 Culley, D. D. and D. E. Ferguson. 1969. Patterns of Insecticide Resistance in the Mosquitofish, *Gambusia affinis*. J. Fish. Res. Board Can 26(9): 2395-2401.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Wild-caught fish from a drainage canal near Belzoni, MS, acclimatized for 1 – 5 days. Fish apparently had fungal infection prior to use and required treatment with malachite green and noniodized table salt. Fish fasted 24-hr prior to testing. Diazinon dissolved in acetone. Test containers were 1-gal jars containing 2.5 l of treatment solution in replicate with 6 fish in each jar (approximately 0.5 g fish/liter).

Limitations of Study: None of the pesticides tested appear to be diazinon or its degradate (diazoxon).

Primary Reviewer: Thomas Steeger, Ph.D., Senior Scientist

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ECOTOX Record Number(s) and Citation: 6221.and **11219** Sancho, E., M. D. Ferrando, E. Andreau and M. Gamon. 1992. Acute Toxicity, Uptake and Clearance of Diazinon by the European Eel, *Anguilla anguilla* (L). J. Envion. Sci. Health. B27(2): 209 – 221.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Wild-caught eels (20 – 30 g; 16 – 20 cm) obtained from Albufera Lake (Valencia, Spain) and acclimated to laboratory conditions for 2 weeks. Eels did not respond to feeding attempts but appeared healthy. Animals were not fed during the 96-hr toxicity study. Diazinon (95% ai) prepared in acetone and presumably diluted with tap water. Glass aquaria (40 l) containing 35 l of test solution; solvent control run with 65 µl acetone/l. Ten eels per replicate and four replicates per treatment were tested.

	24-hr	48-hr	72-hr	96-hr
European eel	160 µg/L	110 µg/L	90 µg/L	80 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of eels to diazinon.

Limitations of Study: Prior chemical exposure (other than diazinon) history is unknown; animals would have been fasted for roughly 3 weeks and likely have mobilized fat reserves where chemical residues may have been present although study claims that diazinon was not detected in the eel prior to exposure.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 7004 and 11438 Sancho, E., M. D. Ferrando, E. Andreu and M. Gamon. 1993. Bioconcentration and Excretion of Diazinon by Eel. Bull. Enviorn. Contam. Toxicol. 50: 578 – 585.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Wild-caught eels (20 – 30 g; 16 – 20 cm) obtained from Albufera Lake (Valencia, Spain) and acclimated to laboratory conditions for 2 weeks. Eels did not respond to feeding attempts but appeared healthy. Animals were not fed during the 96-hr toxicity study. Diazinon (95% ai) prepared in acetone and presumably diluted with tap water. Glass aquaria (40 l) containing 35 l of test solution; solvent control run with 66 µl acetone/l. Ten eels per replicate and four replicates per treatment were tested.

	24-hr	48-hr	72-hr	96-hr
European eel	160 µg/L	110 µg/L	90 µg/L	80 µg/L

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of eels to diazinon.

Limitations of Study: Prior chemical exposure (other than diazinon) history is unknown; animals would have been fasted for roughly 3 weeks and likely have mobilized fat reserves where chemical residues may have been present although study claims that diazinon was not detected in the eel prior to exposure. Essentially the same reference/study as #**6221** and #**11055.**

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 66119. Parkhurst, M A., G. Whelan, Y. Onishi and A. R. Olsen. 1981. Simulation of the Migration, Fate and Effects of Diazinon in two Monticello Stream Channels. Battelle, Pacific Northwest Laboratories Report to the U. S. Army Medical Bioengineering Laoboratory, Fort Dietrick, Frederick, MD. Contract 2311104483.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Only secondary data are cited in the document (Table 3.14). According to the document, the Monticello Experimental Research Station (MERS) borrowed "extensively" from data they had gathered. The primary sources of data are

ECOTOX Record Number and Citation: Sparling, D. W. and G. Fellers. 2006 Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. Environmental Pollution (Article in Press; available online at www.sciencedirect.com).

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Wild-caught foothill yellow-legged frog (*Rana boylii*) egg masses (3) collected from a Coast Range stream. Eggs hatched under laboratory conditions in 78 L aquaria for several weeks prior to test initiation. During acclimation, larvae fed boiled organic romaine lettuce and high-protein fish flakes *ad libitum*.

Chloropyrifos, diazinon and malathion and their respective oxons were reagent grade (99% pure) and purchased from Arco Organics (Morris Plains, NJ). Chemicals were dissolved in acetone. Aquaria (8 L) filled with 7 L of reconstituted water; treatment concentrations are nominal. To each aquarium, 9 "same-aged" *R. boylii* tadposles ranging in developmental stage from Gosner 32 to 44. After the first 24 hr of exposure, tadpoles were fed a small amount of organic romaine lettuce.

Total cholinesterase activity determined via a colormetric method of Ellmann *et al* (1961)[1]. Cholinesterase levels were normalized to that of a metamorph by multiplying by 2.4, 1.9 and 1.6 for tadpoles falling into stages 32 – 36, 37 – 39 and 40 – 45, respectively, to account for what the authors claim is an increase in chloinesterase activity with developmental stage of tadpoles.

Probit dose-response curve results for chlorpyrifos, diazinon, malathion and their respective oxygen analogs (oxons) in *R. boylii*.

Chemical	Period	Slope	LC_{50}	95% Confidence Interval
Chlorpyrifos	24	17.018	3.005	0.993 – 157
Diazinon	96	3.374ns	7.488	NA
Diazoxon	96	14.077	0.760	0.336 – 3.212
Malathion	96	31.477 ns	2.137	NA
Maloxon	96	133.659	0.023	0.014 – 0.180

ns not significant
NA – not available

Regression results of normalized cholinesterase acitivty against concentration for chlorpyrifos, diazinon, malathion and their respective oxygen analogs (oxons) in *R. boylii*.

Chemical	N	Slope	Intercept	R2
Chlorpyrifos	46	-0.0330	0.8499	0.1383
Chloroxon	9	-26.8088	1.2525	0.2547
Diazinon	20	-0.0796	1.2169	0.1729
Diazoxon	45	-0.0511	0.8504	0.0908
Malathion	28	-0.1028	1.0534	0.2244
Maloxon	27	-24.5409	1.0193	0.1557

The study concludes that each pesticide and their respective oxons significantly depressed normalized cholinesterase activity compared to controls. Regressions of normalized cholinesterase activity over exposure concentration indicated that the oxon forms had steeper declines in AchE activity by concentration than their respective parental forms. Maloxon and chloroxon had steeper negative slopes than diazoxon. For the parent compounds, chlorpyrifos decreased AchE activity more rapidly than did malathion (p=0.0201).

The median 96-hr lethal concentrations for each of pesticides studied along with their respective oxons are reported in Table XX. The median 96-hr LC_{50} value for diazinon and diazoxon are 7.49 and 0.76 mg/l, respectively, based on nominal concentrations.

Table 96-hr median lethal concentrations and 95% confidence intervals for organophosphate insecticides and their respective oxygen analogs (oxons); probit dose response slopes and associated probability levels are also reported

	Slope	P of slope	LC_{50} (mg/l)	95% Confidence Interval (mg/l)
Chlorpyrifos	17.02	0.0339	3.005	0.993 – 157

[1] Ellman, G. I. , K. D. Coutney, F. Andres, and R. M. Featherstone. 1961. A new and rapid colorimetric determination of actrylcholinesterase activity. Biochemistry and Pharmacology 7: 88 – 95.

Diazinon	3.374	NS	7.488	NA
Diazinon oxon	14.08	0.001	0.760	0.336 – 3.212
Malathion	31.48	NS	2.137	NA
Malathion oxon	133.7	0.011	0.023	0.014 – 0.180

Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: Study provides useful information on the relative sensitivity of amphibians to diazonon compared to surrogate fish species. Also, the study provides useful information on the toxicitiy of the diazoxon degradate relative to the parent compound.

Limitations of Study: Study relies on nominal concentrations rather than measured; wild-caught animals are used and prior chemical exposure history is unknown.

Peer Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 84407 Lower, N. and A. Moore. 2003. Exposure to insecticides inhibits embryo development and emergence in Atlantic salmon (*Salmo salar* L.). Fish Physiology and Biochemistry 28: 431 – 432.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Six groups of 600 unfertilized eggs placed in 500 ml glass containers and mixed with milt from six male salmon and 200 ml solution with 0.05 and 0.1 μg/L of either cypermethrin or diazinon as well as one group with cypermethrin and diazinon combined at 0.05 μg/L was added. After 2 minutes, the eggs were rinsed in clean water and placed in separate artificial redds.

Fewer fry successfully hatched following exposure to 0.05 and 0.10 μg/L cypermethrin and 0.05 μg/L diazinon compared to other treatment groups. Exposure to 0.05 μg/L cypermethrin caused fry to emerge earlier and exposure to 0.05 μg/L diazinon caused dry to emerge later compared to controls. Disruption of the normal pattern of emergence was greater ($p<0.01$) when embryos were exposed to the pesticides separately, rather than in combination.

Description of Use in Document: Qualitative

Rationale for Use: Study not used quantitatively since exposure concentrations are [presumably] based on nominal and the purity of the test compound is not stated

Limitations for Use: The source of the eggs and male fish used for milt is not specified.; purity of the pesticides is not stated. Concentrations presumed to be nominal since there is no discussion on whether concentrations were measured. No raw data are provided; data are plotted on a graph; however, it is not possible to accurately distinguish treatment groups from the graph. Percent changes in hatch and emergence cannot be determined from the information presented in the paper.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist.

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ECOTOX Record Number and Citation: 53845 Sánchez, M., M. D. Ferrando, E. Sancho and E. Andreu. 1999. Assessment of the toxicity of a pesticide with a two-generation reproduction test using *Daphnia magna*. Comparative Biochemstry and Physiology Part C 124: 247 – 252.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Waterfleas, *Daphnia magna*, obtained from in-house culture. Diazinon (96%) dissolved in acetone. Daphnids (<24 hrs old) exposed during 21 days to 5 diazinon concentrations (0.05, 0.1, 0.5, 0.75 and 1.0 ng/L plus an acetone control (10-4 µl/l). Daphnids housed individually in 60-ml glasss beakers containing 50 ml test solution under static-renewal (24 hr) conditions. Dilution water was dechlorinated tap water. Test animals fed with algae (*N. oculata*). A total of 15 replicates per each treatment. From the first brood (F_1), 15 neonates (<24 hrs old) individually transferred to 60-ml beakers containing clean, untreated water plus solvent control plus negative control and exposed to same concentrations of diazinon as the parents. Afterward, 15 neonates from the third brood (24 hr old) of the parental generation (F_0) from each pesticide exposure concentration individually transferred to 60-ml beakers containing 50 ml toxicant-free solution, plus the controls; the offspring from this third brood were not exposed to diazinon.

Size (body length), fecundity and survival of each generation determined after 21 days of exposure. Longevity, time to the first reproduction, total number of neonates per female, number of broods and brood size, were the criteria used. Neonates were counted daily and then discarded. The intrinsic rate of natural increase (r) was calculated using the following equation: $\Sigma l_x\ m_x\ e\text{-}r_x\text{=}l$ where lx is the proportion of individuals surviving to age x,; m_x is the age-specific number of neonates produced per surviving female at age x (fecundity) and x is days.

Report cites a 24-hr LC_{50} value of 0.86 (0.76 – 0.96) µg/l; however, no data are provided to support this conclusion.

Acccording to the study results summarized in Table XX, length, longevity and number of young per females were significantly different than controls in all of the diazinon treatments. Based on information contained in study tables, longevity of parental generation significantly decreased by 20% in the 0.05 ng/l treatment while number of young decreased by 21% compared to the neat control.

Similarly, brood size, number of young per female and number of broods per female also declined significantly in the F1 generation. Survival decreased by 15% while number of young per female and number of broods per females both declined by 36% and 22%, respectively, relative to controls. These data indicate that the chronic NOAEC for diazinon is less than the lowest concentration tested (<0.05 ng/l) following a 21-day exposure for both parental and F1 generations.

No-observed adverse effect concentration in ng/l for parental (F_0), first brood (F_1 first) and third brood (F_1 third). F_0 exposed to diazinon continuously for 21 days.

Generation	Carapace Length	Longevity	Days to 1st brood	Number of young per female	Brood size	Number of broods per female	r
F_0	<0.05	<0.05	0.1	<0.05	0.05	0.05	0.5
F_1 (first)	<0.05	0.5	0.75	0.1	0.1	0.5	0.5
F_1 (third)	<0.05	0.5	0.5	0.5	0.5	0.5	0.1

Description of Use in Document: Qualitative

Rationale for Use.: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon on a chronic exposure basis.

Limitations of Study: presumably the results are reported in terms of active ingredient. Although the study reports that analytical analyses were conducted, the results of those analyses are not presented and the report simply states that mean measured concentrations were >90% of nominal. It is also uncertain whether statistical analyses were conducted relative to the neat control, the solvent control or the pooled controls. Direct comparisons are made between treated groups and the neat (blank) control so presumably controls were not pooled. In the comparisons for various parameters from the third brood of the first generation daphnia, carapace length, number of young per female and brood size were all significantly different for the solvent control versus the negative control. For number of young per female, the acetone control was 37% larger than the negative control and indicates that the solvent may be having an effect. The study is of questionable utility given that the solvent is having a significant effect. Additionally, the study alludes to the fact that diazinon concentrations are measured; however, the level of detection is not stated. The treatment concentrations of as low as 0.05 ng/L are relatively challenging to detect.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 22702. Sánchez, M., M. D. Ferrando, E. Sancho and E. Andreu. 2000. Physiological Perturbations in Several Generations of *Daphnia magna* Straus Exposed to Diazinon. Ecotoxicology and Environmental Safety 46: 87 – 94

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: This study appears to be identical to Sánchez *et al*. 1999 (**53845)**

Description of Use in Document: Qualitative

Rationale for Use.: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon on a chronic exposure basis.

Limitations of Study: presumably the results are reported in terms of active ingredient. Although the study reports that analytical analyses were conducted, the results of those analyses are not presented and the report simply states that mean measured concentrations were >90% of nominal. It is also uncertain whether statistical analyses were conducted relative to the neat control, the solvent control or the pooled controls. Direct comparisons are made between treated groups and the neat (blank) control so presumably controls were not pooled. In the comparisons for various parameters from the third brood of the first generation daphnia, carapace length, number of young per female and brood size were all significantly different for the solvent control versus the negative control. For number of young per female, the acetone control was 37% larger than the negative control and indicates that the solvent may be having an effect. The study is of questionable utility given that the solvent is having a significant effect. Additionally, the study alludes to the fact that diazinon concentrations are measured; however, the level of detection is not stated. The treatment concentrations of as low as 0.05 ng/L are relatively challenging to detect.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: 71888 Banks, K. E., S. H. Wood, C. Matthews, K. A. Thuesen. 2003. Joint acute toxicity of diazinon and copper to *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 22(7): 1562 – 1567.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Diazinon (99.8% ai) prepared in reconstituted hard water. Ceriodaphnia dubia neonates (<24 hr old) obtained from cultures maintained at the University of North Texas (Denton, TX). Cultures maintained in hard water and fed green algae

(*Pseudokirchneriella subcapitata*), blended trout chow and Cerophyll® (Ward's Natural Science Establishment, Rochester, NY) and were exposed to a 16:8 light:dark photoperiod. Nominal diazinon test concentrations were 0.05, 0.10, 0.20, 0.40 and 0.80 µg/L.

Toxicity tests are reported to have followed procedures recommented by U.S. EPA. Exposures conducted in 30-ml plastic containers filled with 15 ml of test solution. Four replicates each containing 5 neonates used for each treatment. The test was conducted under static conditions and no food was provided to the organisms during the 48-hr test duration. All tests conducted at 25 + 1°C.

The initial concentration of diazinon in the stock solution determined with ELISA (EnviroGard 96 Well Plate Kit.

Control survival was >90% and water quality remained within the guidelines established by EPA (temperature 25±1°C; DO 8.27±0.06 mg/L; pH 8.35 – 8.36; alkalinity 136±9.5 mg/L. The measured concentration of diazinon was within 90% of nominal at test initiation. The 48-hr LC_{50} value was 0.45 µg/L (95% CI: 0.36 – 0.57 µg/L).

Description of Use in Document: Qualitative

Rationale for Use: Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

Limitations of Study: study appears to be scientifically sound; however, it relies on nominal concentrations beyond the single measured concentration on the stock solution.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: Dutta, H. M. and H. J. M. Meijer. 2003. Sublethal effects of diazinon on the structure of the testis of bluegill, *Lepomis macrochirus*: a microscopic analysis. Environmental Pollution 125: 355 – 360.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 2, 2007

Summary of Study Findings: Male adult bluegills were obtained from a fish hatchery near Baltic, OH.; fish were acclimated in the lab for 4 months prior to the study in dechlorinated tap water. Test water quality consisted of 21±1oC, pH 7 ± 0.16; DO 8.27 ± 0.33 mg/L; alkalinity 41.78 ±1.48 mg/L. Fish were fed daily using Tetra Doro Min (Tetra Werke, Germany). Fish were exposed to 60 µg/L for 24, 48, 72 and 96 h and 1 and 2 wk intervals using formulated end-product (25% a,i. 57% aromatic petroleum derivative solvent and 18% inerts. Exposures conducted in 180-l glass tanks under static renewal conditions with water changes every 24 hours. Ten fish were used in a control tank and presumably the same number was in the treatment tank.

After exposure to 24, 48, 72 96 h and 1 and 2 weeks, treated and control fish were euthanized with 100 mg methyltricaine sulfonate/L buffered with 100 mg sodium bicarbonate/L. Average length, body weight and testicular weight recorded. Testes were fixed in Bouin solution for 24 hrs. Diameter measurement (40) were made of seminiferous tubules, the lumen within the tubules and of the spermatogonia and spermatozoa randomly from the control group and the diazinon-treated group at the different exposure periods using an ocular micrometer.

Table 1 summarizes the results of the study. The authors concluded that in the 96 hr group there were significant reductions in both the lumen and seminferous tubule size in comparison with controls and 24, 48 and 72 hr exposures. After 2 weeks of exposure hardly any lumen was seen. The change in the diameter of the seminiferous tubules was very irregular and there was no correlation between the size of the fish, body weight and weight of the testes after different expsoure periods to diazinon. The authors note significant changes in germ cell diameter; however, they do not appear to be consistently correlated with exposure period.

Description of Use in Document: Invalid

Rationale for Use: Potential solvent effect not accounted for**.**

Limitations of Study: the study only tested a single concentration of diazinon. The study measured the response from a formulated product; however, the study cannot distinguish between the effects that may have been due to the organic solvent/inerts co-formulated with the active ingredient.

Table Summary of mean lumen diameter, mean seminiferous tubule lumen diameter, mean germ cell diameter and mean spermatozoa diameter in mm following 24, 48, 72, 96 hr and 1 and 2 week exposures to diazinon formulated endproduct at 60 µg/L.

Treatment	Mean lumen diameter (mm)	Mean seminiferous tubule lumen (mm)	Mean germ cell diameter (mm)	Mean spermatozoa diameter (mm)
Control	0.01878	0.0647	0.0129	0.001994
24 hr	0.0343 b	0.0836 b	0.0134	0.001875
48 hr	0.0142 a	0.058	0.0112 a	0.001769 a
72 hr	0.0485 b	0.0849 b	0.0126	0.001694 b
96 hr	0.0072 b	0.0514 a	0.0104 b	0.00124 b
1 week	0.0218 a	0.0692	0.0095 b	0.001575 b
2 week	0.0081 b	0.0528 b	0.0094 b	0.001638 b

a Significant
b Highly Significant

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

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ECOTOX Record Number and Citation: Banks, K. E., P. K. Turner, S. H. Wood, and C. Matthews. 2005. Increased toxicity to *Ceriodaphnia dubia* in mixtures of atrazine and diazinon at environmentally realistic concentrations. Ecotoxicology and Environmental Safety 60: 28 – 36.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: March 30, 2007

Summary of Study Findings: Diazinon (99.8% ai) prepared in reconstituted hard water. Ceriodaphnia dubia neonates (<24 hr old) obtained from cultures maintained at the University of North Texas (Denton, TX). Cultures maintained in hard water and fed green algae (*Pseudokirchneriella subcapitata*), blended trout chow and Cerophyll® (Ward's Natural Science Establishment, Rochester, NY) and were exposed to a 16:8 light:dark photoperiod. Nominal diazinon test concentrations were 0.10, 0.20, 0.40, 0.6, 5, 10, 20 and 40 µg/L.

Toxicity tests are reported to have followed procedures recommented by U.S. EPA. Exposures conducted in 30-ml plastic containers filled with 15 ml of test solution. Four replicates each containing 5 neonates used for each treatment. The test was conducted under static conditions and no food was provided to the organisms during the 48-hr test duration. All tests conducted at 25 + 1^{o}C.

The initial concentration of diazinon in the stock solution determined with ELISA (EnviroGard 96 Well Plate Kit.

Control survival was $\geq$90% and water quality remained within the guidelines established by EPA (temperature 25$\pm$1^{o}C; DO 8.27$\pm$0.06 mg/L; pH 8.35 – 8.36; alkalinity 136$\pm$9.5 mg/L. The measured concentration of diazinon was within 90% of nominal at test initiation. The 48-hr LC_{50} value was 0.21 µg/L (95% CI: 0.17 – 0.25 µg/L).

The study also notes that in combination with atrazine ranging from 5 to 40 µg/L, diazinon 48-hr LC_{50} values were lower (more sensitive) than with diazinon alone.

Table Median lethal concentrations for diazinon alone and in combination with increasing concentrations of atrazine.

	LC_{50} and 95% Confidence Interval (µg/L)
Diazinon alone	0.21 (0.17 – 0.25)
Diazinon + 5 µg/L atrazine	0.16 (0.14 – 0.19)
Diazinon + 10 µg/L atrazine	0.12 (0.11 – 0..15)

Diazinon + atrazine	20	μg/L	0.14 (0.12 – 0.16)
Diazinon + atrazne	40	μg/L	0.13 (0.11 – 0.16)

Description of Use in Document: Quantitative
Rationale for Use: Study is appears to be scientifically sound and provides a more sensitive endpoint on acute diazinon toxicity to freshwater invertebrates than is available through registrant-submitted data.

Limitations of Use: study appears to be scientifically sound; however, it relies on nominal concentrations beyond the single measured concentration on the stock solution. The depression in median lethal concentrations for diazinon when in combination with atrazine does not appear to be concentration dependent.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

Secondary Reviewer: Kristina Garber, Biologist

ECOTOX Record Number and Citation: 62247. Scholz, N. L., N. K. Truelove, G. L. French, B. A. Berejikian, T. P. Quinn, E. Casillas and T. K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Science 57: 1911 – 1918.

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OFFICE OF
RESEARCH AND DEVELOPMENT

February 22, 2001

MEMORANDUM

SUBJECT: Review of papers on diazinon effects on salmon olfaction

FROM: Dave Mount ORD/NHEERL/MED

TO: Tom Steeger OPPTS/OPP/EFED

At your request, I have reviewed two manuscripts regarding the effects of diazinon on olfaction in salmon. These are:

Scholz, N.L., N.K. Truelove, B.L. French, B.A. Berejikian, T.P. Quinn, E. Casillas, and T.K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 57:1911-1918.

Moore, A., and C.P. Waring. 1996. Sublethal effects of the pesticide diazinon on olfactory function in the mature male Atlantic salmon parr. J. Fish. Biol. 48:758-775.

The Moore and Waring paper deals with electrophysiological measurements on the olfactory epithelium of salmon and on olfactory-stimulated hormone production in salmon, both after exposure to waterborne diazinon. In general I found no obvious faults with the experimental procedures. The electrophysiological experiments used repeated measures on the same fish and I didn't see any data in the paper to show that this is not an issue, although the text indicates reference measurements were made to determine the effect of this procedure. The olfactory responses were made relative to a standard exposure to L-serine; I'm not familiar with this procedure so I can't comment on how to interpret the absolute values of the responses. Some of the graphs also don't make clear what the control response was (e.g., Figure 1), leaving unclear what effect the lowest exposures had relative to control.

Details aside, the overall package does seem to suggest that olfactory responses of salmon measured in this way (electrophysiogram of perfused olfactory rosettes) are changed by exposure to increasing concentrations of diazinon. The interpretation of these effects is discussed farther below.

The second portion of the Moore and Waring paper evaluates the stimulation of several hormones in male parr exposed to female salmon urine with or without pre-exposure to diazinon. Again, I have some minor quibbles with the procedures and data presentation. An exposure to industrial methylated spirits (IMS) alone, without urine, would have been useful. Also, the data analysis seems confused (figs 4 and 5); rather than determining whether the response was significantly greater than the negative control (no urine), in seems much more logical to determine whether the response with diazinon exposure was significantly reduced from the positive control. On balance, however, it does not seem unreasonable to conclude that exposure to diazinon at some concentration changes response to priming with female salmon urine when measured in this way.

The Scholz et al. paper also contains experiments of two types: 1) effects of diazinon pre-exposure on responses to an "alarm" stimulus (a water extract of homogenized salmon skin); and 2) return of salmon to the source hatchery after pre-exposure to varying concentrations of diazinon. In the first set of experiments, individual young salmon are exposed to one of several concentrations of waterborne diazinon for 2 hours, then returned to an observation tank where their activity and feeding behavior (on live daphnids) is monitored for 8 minutes, then a standard aliquot of skin extract is introduced, followed by another 8 minutes of observation. The negative control response is for an approximately 80% reduction in activity and about 90% reduction in food strikes following introduction of the skin extract, presumably indicating a natural response to predation occuring in the field. Following on the work of Moore and Waring, if diazinon affects olfaction, then this "alarm response" would be reduced following diazinon exposure.

The data from these experiments indicate that the 2-hour diazinon pre-exposure did not have an effect on activity or feeding behavior <u>prior</u> to introduction of the skin extract. After introduction of the skin extract, activity and feeding behavior was reduced in all treatments and control; however, the magnitude of the response was significantly reduced (or nearly so) in fish pre-exposed to diazinon at 1 ug/L or 10 ug/L. It should be noted that this "alarm" response was not eliminated, only reduced. For example, in control fish, the post-extract activity was reduced by about 82% from pre-extract activity, while after 10 ug/L pre-exposure, post-extract activity was reduced by about 68%.

The homing study evaluated the effect of diazinon on the ability of fish that had already returned to their natal hatchery to return after being transplanted from the hatchery back to a downstream (2 km) location. At total of 40 fish in each of four treatment groups (control and 0.1, 1.0, and 10 ug/L diazinon pre-exposure) were released downstream; of these, a total of 16, 12, 12, and 6 fish, respectively, returned to the hatchery and were recaptured. The statistical tests applied by the authors find that the return of 6 fish in the highest diazinon treatment was significantly different from the solvent control. The design of this experiment causes some discomfort; one could argue that treating the individual fish as the sampling unit is a form of pseudoreplication. Furthermore, the fish were actually released in a series of small groups, but the details are vague and the results are only given in "lump" form. It seems possible that the individual release dates could be used as an experimental unit instead of the individual fish, but this was not done for some reason. The design in general is not very robust; it would be strengthened greatly if the entire experiment would be repeated. The authors also note that the return rate for the control fish was inexplicably lower than has been observed for similar releases in previous years, although the impact of that on the findings is not immediately obvious. Overall, it seems more likely than not that there may be some effect here, but this is by far the weakest of the experiments in terms of experimental design and interpretation. This is unfortunate, since it is the study that most closely links to assessment endpoints likely to be of concern for ecological risk assessments for this species.

In summary then, all of these experiments (with the possible exception of the last) seem to demonstrate a statistically significant change in physiology or behavior that can be at least theoretically tied to effects of diazinon on olfaction in salmon. The primary issue is how to interpret this information in the context of ecological risk assessment, which is the focus of the remaining discussion. For expediency, I'll refer to the four sets of experiments as the "epithelial", "priming", "alarm", and "homing" studies (in the order described above).

I presume that Agency risk assessments to which these data might be applied would have as their assessment endpoint something like, "protection of balanced, indigenous aquatic communities," or perhaps, "maintenance of naturally reproducing salmon populations." The basic difficulty in interpreting these studies in the context of ecological risk is that the measurements that are made (particularly in the epithelial, priming, and alarm studies) are not clearly tied to these assessment endpoints. One can easily develop scenarios where it is plausible that these measures might affect salmon at the population level, but it is also possible that these changes might be compensated for in other ways that would result in no effect on the population. There is no quantitative link established between these responses and changes in a field population. The Agency's *Framework for Ecological Risk Assessment* (1992) identifies this problem:

> In many cases, measurement endpoints at lower levels of biological organization may be more sensitive than those at higher levels. However, because of compensatory mechanisms and other factors, a change in a measurement endpoint at a lower organizational level (e.g., a biochemical alteration) may not necessarily be reflected in changes at a higher level (e.g., population effects). (p. 14)

And later on:

> Ideally, the stressor-response evaluation quantifies the relationship between the stressor and the assessment endpoint. When the assessment endpoint can be measured, this analysis is straightforward. When it cannot be measured, the relationship between the stressor and measurement endpoint is established first, then additional extrapolations, analyses, and assumptions are used to predict or infer changes in the assessment endpoint. (p. 23)
>
> Measurement endpoints are related to assessment endpoints using the logical structure presented in the conceptual model. In some cases, quantitative methods and models are available, but often the relationship can be described only qualitatively. Because of the lack of standard methods for many of these analyses, professional judgement is an essential component of the evaluation. It is important to clearly explain the rationale for any analyses and assumptions. (p. 23)

Ambient Water Quality Criteria (AWQC) to protect aquatic life represent one of relatively few attempts to standardize the use of toxicity data in risk assessments. The guidelines for deriving these criteria (Stephan et al., 1985) focus on toxicity test endpoints that have direct applicability to population demographics – basically, survival, growth, and reproduction. Other effects are not considered unless there is strong evidence of a direct link between the measured endpoint and survival, growth, or reproduction. In general, data such as those generated by the epithelial, priming, and alarm studies would not be considered directly in the criteria derivation.

Existing criteria documents contain many types of data that were not used in the criteria derivation (the documents collate and review these data, but they are not used to actually define the criterion concentration). For example, behavioral studies with copper and other chemicals have shown avoidance behavior in the laboratory at very low concentrations (e.g., rainbow trout will avoid 1 ug Cu/L). While one could imagine this affecting populations in the field, it is also reasonable to expect that many top notch trout fisheries have ambient copper concentrations of at least 1 ug/L. Presumably, other compensatory factors keep the behavioral response measured under laboratory conditions from resulting in noticeable population-level impacts.

Histological or biochemical changes are often reported for many chemicals at concentrations below that shown to directly affect survival, growth, or reproduction in laboratory toxicity tests. These might be more similar to the epithelial studies conducted by Moore and Waring. The recent revision of the ammonia criteria document (accessible through the OW/OST website) has the following to say about the use of histological endpoints:

> Endpoint indices of abnormalities such as reduced growth, impaired reproduction, reduced survival, and gross anatomical deformities are clinical expressions of altered structure and function that originate at the cellular level. Any lesion observed in the test organism is cause for concern and such lesions often provide useful insight into the potential adverse clinical and subclinical effects of such toxicants as ammonia. For purposes of protecting human health or welfare these subclinical manifestations often serve useful in establishing 'safe' exposure conditions for certain sensitive individuals within a population.
>
> With fish and other aquatic organisms the significance of the adverse effect can be used in the derivation of criteria only after demonstration of adverse effects at the population level, such as reduced survival, growth, or reproduction. Many of the data indicate that the concentrations of ammonia that have adverse effects on cells and tissues do not correspondingly cause adverse effects on survival, growth, or reproduction. No data are available that quantitatively and systematically link the effects that ammonia is reported to have on fish tissues with effects at the population level. This is not to say that the investigators who reported both tissue effects and population effects within the same research did not correlate the observed tissue lesions and cellular changes with effects on survival, growth, or reproduction, and ammonia concentrations. Many did, but they did not attempt to relate their observations to ammonia concentrations that would be safe for populations of fish under field conditions nor did they attempt to quantify (e.g.,

increase in respiratory diffusion distance associated with gill hyperplasia) the tissue damage and cellular changes (Lloyd 1980; Malins 1982). Additionally, for the purpose of deriving ambient water quality criteria, ammonia-induced lesions and cellular changes must be quantified and positively correlated with increasing exposures to ammonia.

In summary, the following have been reported:
1. Fish recover from some histopathological effects when placed in water that does not contain added ammonia.
2. Some histopathological effects are temporary during continuous exposure of fish to ammonia.
3. Some histopathological effects have occurred at concentrations of ammonia that did not adversely affect survival, growth, or reproduction during the same exposures.

Because of the lack of a clear connection between histopathological effects and effects on populations, histopathological endpoints are not used in the derivation of the new criterion, but the possibility of a connection should be the subject of further research.

In human health risk assessment, deviations from normal physiology are generally considered to be adverse effects. As described in the text from the ammonia document, the practice in AWQC and in other ecological risk assessments in general, is to focus on effects that cause changes at the population level; this requires the ability to make this link in a manner quantitative enough to say how strong a response in the measured parameter would adversely effect populations.

The combined evidence from the Moore and Waring and Scholz et al. studies do not clearly provide this connection. The electrophysiograph data from the epithelial studies provide strong evidence that diazinon exposure can induce measurable changes in activity of the epithelial rosettes, but there are no means to connect this directly to changes in survival, growth, or reproduction. As shown in Figures 1 and 2 of Moore and Waring, diazinon exposure produces a concentration-dependent decrease in rosette responsiveness, but responsiveness is not lost, just reduced. Thus, the question becomes, “What is the minimum level of rosette activity necessary?”

The priming studies performed by Moore and Waring provide a closer link to reproductive success; these studies link diazinon exposure to changes in reproductive hormone response to priming with female salmon urine. However, data for the endpoint most directly related to reproduction, milt production, were equivocal. The data (figure 6) show a significant increase in milt production in fish primed with urine or urine plus carrier solvent relate to unstimulated fish. However, the more relevant question would be whether diazinon treatment decreases milt production relative to the solvent control; this comparison isn’t made, but it does not appear likely that is did, based on the figure. Further, even if one concludes that there is an effect in milt release under these conditions, it isn’t clear whether this would actually affect reproductive success under field conditions.

The alarm response studies show a decrease in the so-called “alarm response” following pre-exposure to diazinon, and the nature of this response is consistent with what might be expected based on the olfactory effects shown by Moore and Waring. While a significant change was found, a substantial alarm response was still present in diazinon-exposed fish. Whether the degree of change noted is sufficient to affect survival/growth/reproduction in the field is uncertain.

The homing studies provide data that are closest to making the link to effects on populations. Clearly, relatively little supposition or extrapolation is necessary to infer that reduced migratory capability could have adverse effects on salmon populations. There is still some question about “how much is too much”, but not substantially more so than is faced in interpreting ordinary survival or growth data. Unfortunately, this study is compromised somewhat by a weak design and lack of replication. Having further data on this response using a more robust design (e.g., releasing several lots of fish over the course of several days) would be helpful.

Judging the significance of any of these findings in producing ecological risk is also dependent on determining the relationship between actual exposures that are observed in the field. Although the authors claim that they occur, pulses of diazinon to 10 ug/L are not something that occurs very often to my knowledge – this seems extreme.

Also relevant is how to interpret the likely effects of field exposures on the aquatic community in general. In a construct like AWQC, the much greater sensitivity of other organisms, such as cladocerans (toxic effects in the 0.1 ug/L range), to diazinon cause “acceptable risk” to be exceeded at diazinon concentrations below those showing significant effects on salmon olfaction. This approach doesn’t get at how to deal quantitatively with the olfaction data, it just makes it moot for diazinon. If the assessment endpoint is populations of salmon *per se*, rather than protection of aquatic communities, then the problem doesn’t go away, unless one considers cladocerans and other organisms highly sensitive to diazinon as part of the habitat essential to maintain salmon populations (after all, it takes more than just water to maintain salmon).

One of the questions you posed was in regard to a desire from the Services to include the alarm response assay as a standard screening test. Two things would generally be required: 1) that the test is shown to be sufficiently reproducible within and between laboratories; and 2) that the endpoint of the assay be more sufficiently tied to the assessment endpoint (presumably maintenance of salmon populations or aquatic communities). If one were to attempt the latter, it would seem that combining the olfaction assays with the homing studies for multiple chemicals in multiple trials would be a good first step, though I don’t know how reliable it is to assume that something that blocks the alarm response would necessarily interfere with homing (or the reverse). If no more attempt is made to relate the olfaction assays with populations response, it will be very difficult to move the olfaction issue into a part of the risk calculation rather than being simply a component of the qualitative uncertainty.

I’ve spent most of this discussion describing things that discourage the use of these data in quantitatively describing risk. I should counter this by saying that the difficulty of incorporating this information into a risk assessment should not be taken to suggest that adverse effects of diazinon on salmon populations are not possible via this mechanism (provided exposures were sufficiently high). Certainly the cluster of studies looking at the issue show a fair amount of internal consistency with regard to the existence of such an effect at concentrations below those that reduce survival or growth in salmon or other fish species. This particular case is even more troubling because it is unlikely that any traditional toxicity test could effectively measure effects on salmon reproduction directly, and, in the case of salmon, successful reproduction in the field is thought/known to be dependent on olfaction in ways that wouldn't be assessed using traditional chronic toxicity tests on this or other fish species. Describing this uncertainty qualitatively within a risk assessment would definitely be appropriate, even if olfaction data are not part of the quantitative risk calculation. The risk manager will be faced with the decision as to how this uncertainty affects management decisions; at this point, I'm not sure that our scientific understanding can do more than frame the question.

Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. EPA, Environmental Research Laboratory, Duluth, MN. NTIS No. PB85-227049. 98 pp.

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Rodgers, M. H. 2005b. Diazoxon (a metabolite of the active ingredient diazinon) Dietary Toxicity (LD_{50}) to the Bobwhite Quail. Huntingdon Life Sciences Limited, Woolley Rd, Alconbury, Huntingdon, Cambridgeshire, England (Huntingdon Project ID: MAK 872). Sponsored by Makhteshim-Agan of North America Inc., 4515 Falls of Neuse Rd., Suite 300, Raleigh, NC 27609 (Makhteshim Project Number: R-18131). Study initiated: 04/05/05; study completed: 05/25/05 (**MRID 465796-02**)

The acute dietary toxicity of diazoxon, a metabolite of th active ingredient diazinon, to approximately 12-d old Bobwhite quail (*Colinus virginianus*) was assessed over 8 days (5 days of exposure plus 3-day post-exposure observation period). Diazoxon was administered to the birds in the diet at 30, 60, 120, 240, 480 and 960 mg a.i/kg diet of diet. The 5 day acute dietary LC_{50} was 72.3 mg a.i/kg of diet. The 5-day NOAEC of diazoxon based on reduced body weight was 9.4 mg a.i/kg diet of diet (based on a preliminary study). According to the US EPA classification, diazoxon would be classified as highly toxic to Bobwhite quail on a subacute dietary exposure basis.

Clinical signs were confined to unsteadiness/inability to stand and subdued behavior in the groups treated with at 60, 120, 240, 480 and 960 mg/kg diet. All birds in the groups treated at 60, 120, 240, 480 and 960 mg/kg diet displayed clinical and/or were found dead. Mortality was observed at 60 (20%), 120 (100%), 240 (100%), 480 (100%) and 960 (100%) mg/kg diet.

This toxicity study is classified as scientifically sound and is thus acceptable and does satisfy the guideline requirement for subacute dietary toxicity study for Bobwhite quail.

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Rodgers, M. H. 2005a. Diazoxon (a metabolite of the active ingredient diazinon) Acute Oral Toxicity (LD_{50}) to the Bobwhite Quail. Huntingdon Life Sciences Limited, Woolley Rd, Alconbury, Huntingdon, Cambridgeshire, England (Huntingdon Project ID: MAK 874). Sponsored by Makhteshim-Agan of North America Inc., 4515 Falls of Neuse Rd., Suite 300, Raleigh, NC 27609 (Makhteshim Project Number: R-18127) (**MRID 465796-04**).

The acute oral toxicity of diazoxon (a metabolite of the active ingredient diazinon) to 27-wk old Bobwhite quail *(Colinus virginianus*) was assessed over 14 days. Diazoxon was administered to the birds by oral intubation (gavage) at 0.79, 1.31, 2.18,.3.61 and 6.00 mg a.i./kg bw. The 14-day acute oral LD_{50} was 4.94.mg a.i/kg bw. The 14-day NOEL of diazoxon to the Bobwhite quail, based on mortality and behavioral effects was 2.18 mg a.i/kg bw. According to the US EPA classification, diazoxon would be classified as very highly toxic to Bobwhite quail on an acute oral exposure basis.

No clinical signs observed in groups dosed at 0.75, 1.30, 2.25 mg/kg bw or the control group. Clinical signs observed in the groups dosed at 3.63 and 6.16 mg a.i./kg bw were confined to subdued behavior, unsteadiness and frothy fluid around the beak on the day of dosing. No other clinical signs were observed through the remainder of the observation period.

This toxicity study is classified as scientifically sound and is acceptable; the study is consistent with guideline requirements for an acute oral toxicity study using Bobwhite quail.

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Grade, R. 1993a. Report on the acute toxicity of G27550 (Oxypyrimidine) to rainbow trout (*Oncorhynchus mykiss*). Ciba-Giegy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. Project Number 932504. Sponsor: Makhteshim Chemical Works, Ltd., 551 Fifth Ave. Suite 1100, New York, New York 100176. (**MRID 463643-12**).

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to technical grade G 27550 (Oxypyrimidine) at measured concentrations of 0, 9.8, 18.1, 32.3, 60.8 and 101.1mg a.i./L under static conditions. The 96-h LC_{50} was greater than the highest concentration (101.1 mg a.i/L) tested. The NOEC value, based on sub-lethal effects, was 60.8 mg a.i/L. Sublethal effects (swimming behavior, loss of equilibrium, respiratory effects) were observed in the groups exposed to 101.1 mg a.i./L of G27550. Based on the results of this study, G 27550 would be classified as practically nontoxic to rainbow trout in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound; however, because the study was conducted under static conditions and failed to characterize water quality parameters adequately and exceeded recommended ranges for both pH and water hardness, the study is classified as supplemental.

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Grade, R. 1993b. Report on the acute toxicity of G27550 (Oxypyrimidine) on *Daphnia magna*. Ciba-Giegy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. Project Number 932505. Sponsor: Makhteshim Chemical Works, Ltd., 551 Fifth Ave. Suite 1100, New York, New York 100176. (**MRID 463643-13**).

The 48-hr-acute toxicity of the diazinon degradate oxypyrimidine to *Daphnia magna* was studied under static conditions. Daphnids were exposed to control and test chemical measured at 10.2, 18.4, 32.7, 59.3 and 101.6 mg a.i/L for 48 hr. Mortality and sublethal effects were observed daily. The 48- hour LC_{50} was greater than 101.6 mg a.i/L. The 48-hr NOEC based on mortality was 101.6 mg a.i/L. No sublethal effects were observed during the study period.

Based on the results of this study, oxypyrimidine would be classified as practically nontoxic to the freshwater invertebrate *Daphnia magna* in accordance with the classification system of the U. S. EPA.

This study is classified as supplemental and can be upgraded to core if the registrant can demonstrate that neither water hardness and/or pH affect the toxicity and solubility of oxypyrimidine. Additionally, the registrant should provide more information on the quality of water used in the study.

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Grade, R. 1993c. Report on the growth inhibition of G27550 (Oxypyrimidine) to Green Algae (*Scenedesmus suspicatus*). Ciba-Giegy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. Project Number 932507. Sponsor: Makhteshim Chemical Works, Ltd., 551 Fifth Ave. Suite 1100, New York, New York 100176. (**MRID 463643-14**).

In a 72 hour acute toxicity study, the cultures of green algae (*Scenedesmus subspicatus*) were exposed to oxypyrimidine at measured concentrations of 1.1, 3.8, 11.6, 35.2 and 109.1 mg a.i/L under static conditions. The NOAEC or EC_{05} and EC_{50}/IC_{50} values based on cell density were 109.1 mg a.i./L and >109.1 mg a.i./L, respectively. No phytotoxic effects were reported in the study; therefore, there were no compound related phytotoxic effects.

This toxicity study is classified as scientifically sound; however, because of the lack of information regarding the study water, this study is classified as supplemental.

Appendix B. Example output file from PRZM/EXAMS modeling (2 aerial applications to lettuce)

stored as Diaz_lettuceben.out
Chemical: Diazinon
PRZM environment: CAlettuceC.txt modified Monday, 11 October 2004 at 15:23:40
EXAMS environment: pond298.exv modified Thuday, 29 August 2002 at 16:33:30
Metfile: w23273.dvf modified Wedday, 3 July 2002 at 09:04:22
Benthic segment concentrations (ppb)

Year	Peak	96 hr	21 Day	60 Day	90 Day	Yearly
1961	5.042	5.041	5.025	4.913	4.767	2.967
1962	19.79	19.78	19.72	19.29	18.72	11.64
1963	18.84	18.84	18.78	18.37	17.84	11.83
1964	7.919	7.918	7.893	7.714	7.497	5.395
1965	6.293	6.292	6.272	6.125	5.937	4.08
1966	6.918	6.917	6.894	6.736	6.533	4.215
1967	5.987	5.986	5.967	5.851	5.703	3.801
1968	6.492	6.491	6.469	6.315	6.118	3.953
1969	28.35	28.34	28.25	27.61	26.8	16.63
1970	21.19	21.19	21.11	20.6	19.93	12.8
1971	6.948	6.947	6.924	6.776	6.59	4.792
1972	4.369	4.368	4.353	4.248	4.114	2.697
1973	11.72	11.72	11.68	11.42	11.07	6.892
1974	17.92	17.92	17.86	17.44	16.89	10.46
1975	17.7	17.7	17.64	17.25	16.75	10.91
1976	14.82	14.82	14.79	14.49	14.08	9.092
1977	8.5	8.499	8.471	8.3	8.187	5.834
1978	29.09	29.08	28.99	28.33	27.47	16.95
1979	12.61	12.6	12.56	12.29	12	8.379
1980	11.78	11.78	11.74	11.48	11.14	7.149
1981	24.46	24.46	24.38	23.81	23.08	14.38
1982	9.972	9.97	9.938	9.712	9.441	6.766
1983	29.26	29.25	29.15	28.45	27.54	16.85
1984	7.617	7.616	7.59	7.415	7.217	4.919
1985	6.358	6.357	6.338	6.202	6.025	3.911
1986	13.63	13.63	13.58	13.24	12.81	7.853
1987	15.35	15.34	15.29	14.92	14.44	8.982
1988	10.62	10.62	10.58	10.42	10.15	6.484
1989	5.218	5.218	5.198	5.06	4.883	3.126
1990	5.079	5.078	5.059	4.925	4.755	2.948

Sorted results

Prob.	Peak	96 hr	21 Day	60 Day	90 Day	Yearly
0.032258064516129	29.26	29.25	29.15	28.45	27.54	16.95
0.0645161290322581	29.09	29.08	28.99	28.33	27.47	16.85

0.0967741935483871	28.35	28.34	28.25	27.61	26.8	16.63
0.129032258064516	24.46	24.46	24.38	23.81	23.08	14.38
0.161290322580645	21.19	21.19	21.11	20.6	19.93	12.8
0.193548387096774	19.79	19.78	19.72	19.29	18.72	11.83
0.225806451612903	18.84	18.84	18.78	18.37	17.84	11.64
0.258064516129032	17.92	17.92	17.86	17.44	16.89	10.91
0.290322580645161	17.7	17.7	17.64	17.25	16.75	10.46
0.32258064516129	15.35	15.34	15.29	14.92	14.44	9.092
0.354838709677419	14.82	14.82	14.79	14.49	14.08	8.982
0.387096774193548	13.63	13.63	13.58	13.24	12.81	8.379
0.419354838709677	12.61	12.6	12.56	12.29	12	7.853
0.451612903225806	11.78	11.78	11.74	11.48	11.14	7.149
0.483870967741936	11.72	11.72	11.68	11.42	11.07	6.892
0.516129032258065	10.62	10.62	10.58	10.42	10.15	6.766
0.548387096774194	9.972	9.97	9.938	9.712	9.441	6.484
0.580645161290323	8.5	8.499	8.471	8.3	8.187	5.834
0.612903225806452	7.919	7.918	7.893	7.714	7.497	5.395
0.645161290322581	7.617	7.616	7.59	7.415	7.217	4.919
0.67741935483871	6.948	6.947	6.924	6.776	6.59	4.792
0.709677419354839	6.918	6.917	6.894	6.736	6.533	4.215
0.741935483870968	6.492	6.491	6.469	6.315	6.118	4.08
0.774193548387097	6.358	6.357	6.338	6.202	6.025	3.953
0.806451612903226	6.293	6.292	6.272	6.125	5.937	3.911
0.838709677419355	5.987	5.986	5.967	5.851	5.703	3.801
0.870967741935484	5.218	5.218	5.198	5.06	4.883	3.126
0.903225806451613	5.079	5.078	5.059	4.925	4.767	2.967
0.935483870967742	5.042	5.041	5.025	4.913	4.755	2.948
0.967741935483871	4.369	4.368	4.353	4.248	4.114	2.697

0.1 27.961 27.952 27.863 27.23 26.428 16.405

Average of yearly averages: 7.8895

Inputs generated by pe4.pl - 8-August-2003

Data used for this run:
Output File: Diaz_lettuce
Metfile: w23273.dvf
PRZM scenario: CAlettuceC.txt
EXAMS environment file: pond298.exv
Chemical Name: Diazinon

Description	Variable Name	Value	Units	Comments
Molecular weight	mwt	304.3	g/mol	
Henry's Law Const.	henry	1.40e-6	atm-m^3/mol	
Vapor Pressure	vapr	1.40e-4	torr	
Solubility	sol	400	mg/L	
Kd	Kd		mg/L	

Koc	Koc	616	mg/L		
Photolysis half-life	kdp	37	days	Half-life	
Aerobic Aquatic Metabolism	kbacw	77.4	days	Halfife	
Anaerobic Aquatic Metabolism		kbacs	0	days	Halfife
Aerobic Soil Metabolism	asm	38.7	days	Halfife	
Hydrolysis:	pH 5	12	days	Half-life	
Hydrolysis:	pH 7	138	days	Half-life	
Hydrolysis:	pH 9	77	days	Half-life	
Method:	CAM	2	integer	See PRZM manual	
Incorporation Depth:	DEPI	0	cm		
Application Rate:	TAPP	2.24	kg/ha		
Application Efficiency:	APPEFF		0.95	fraction	
Spray Drift	DRFT	0.05	fraction of application rate applied to pond		
Application Date	Date	25-01	dd/mm or dd/mmm or dd-mm or dd-mmm		
Interval 1	interval	30	days	Set to 0 or delete line for single app.	
Record 17:	FILTRA				
	IPSCND	2			
	UPTKF				
Record 18:	PLVKRT				
	PLDKRT				
	FEXTRC	0.5			
Flag for Index Res. Run	IR	Pond			
Flag for runoff calc.	RUNOFF	none	none, monthly or total(average of entire run)		

Appendix C. Example output from TerrPlant v.1.2.2 model (single aerial application to lettuce)

Table 1. Chemical Identity.

Chemical Name	**Diazinon**
PC code	**CARLF**
Use	**lettuce (incorporation)**
Application Method	**aerial**
Application Form	**liquid**
Solubility in Water (ppm)	**40**

Table 2. Input parameters used to derive EECs.

Input Parameter	Symbol	Value	Units
Application Rate	A	**2**	**lbs a.i./A**
Incorporation	I	**1.6**	none
Runoff Fraction	R	**0.02**	none
Drift Fraction	D	**0.05**	none

Table 3. EECs for Diazinon. Units in lbs a.i./A.

Description	Equation	EEC
Runoff to dry areas	(A/I)*R	0.025
Runoff to semi-aquatic areas	(A/I)*R*10	0.25
Spray drift	**A*D**	**0.1**
Total for dry areas	**((A/I)*R)+(A*D)**	**0.125**
Total for semi-aquatic areas	**((A/I)*R*10)+(A*D)**	**0.35**

Table 4. Plant survival and growth data used for RQ derivation. Units are in lbs a.i./A.

	Seedling Emergence		Vegetative Vigor	
Plant type	**EC$_{25}$**	**NOAEC**	**EC$_{25}$**	**NOAEC**
Monocot	**5.26**	**x**	**7**	**x**
Dicot	**9.03**	**x**	**3.23**	**x**

Table 5. RQ values for plants in dry and semi-aquatic areas exposed to Diazinon through runoff and/or spray drift.*

Plant Type	Listed Status	Dry	Semi-Aquatic	Spray Drift
Monocot	non-listed	<0.1	<0.1	<0.1
Monocot	listed	#VALUE!	#VALUE!	#VALUE!
Dicot	non-listed	<0.1	<0.1	<0.1
Dicot	listed	#VALUE!	#VALUE!	#VALUE!

*If RQ > 1.0, the LOC is exceeded, resulting in potential for risk to that plant group.

Appendix D. Example output from T-REX v.1.3.1 model (single application to lettuce)

Summary of Risk Quotient Calculations Based on Upper Bound Kenega EECs

Table X. Upper 90th Percentile Kenaga, Acute Avian Dose-Based Risk Quotients										
Size Class (grams)	Adjusted LD50	EECs and RQs								
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
20	0.75	546.67	731.15	250.56	335.11	307.50	411.27	34.17	45.70
100	0.95	311.74	327.51	142.88	150.11	175.35	184.22	19.48	20.47
1000	1.34	139.57	103.81	63.97	47.58	78.51	58.39	8.72	6.49

Table X. Upper 90th Percentile Kenega, Subacute Avian Dietary Based Risk Quotients								
	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
LC50	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
32	480.00	15.00	220.00	6.88	270.00	8.44	30.00	0.94

Size class not used for dietary risk quotients

Table X. Upper 90th Percentile Kenega, Chronic Avian Dietary Based Risk Quotients								
	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
NOAEC (ppm)	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
8	480.00	57.83	220.00	26.51	270.00	32.53	30.00	3.61

Size class not used for dietary risk quotients

Table X. Upper 90th Percentile Kenaga, Acute Mammalian Dose-Based Risk Quotients												
Size Class (grams)	Adjusted LD50	EECs and RQs										
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Granivore	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
15	1109.90	457.64	0.41	209.75	0.19	257.42	0.23	28.60	0.03	6.36	0.01
35	898.03	316.29	0.35	144.97	0.16	177.91	0.20	19.77	0.02	4.39	0.00
1000	388.43	73.33	0.19	33.61	0.09	41.25	0.11	4.58	0.01	1.02	0.00

Table X. Upper 90th Percentile Kenega, Acute Mammalian Dietary Based Risk Quotients								
LC50 (ppm)	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
0	480.00	#####	220.00	#####	270.00	#####	30.00	#####

Size class not used for dietary risk quotients

Table X. Upper 90th Percentile Kenega, Chronic Mammalian Dietary Based Risk Quotients								
NOAEC (ppm)	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
10	480.00	48.00	220.00	22.00	270.00	27.00	30.00	3.00

Size class not used for dietary risk quotients

Table X. Upper 90th Percentile Kenega, Chronic Mammalian Dose-Based Risk Quotients											
Size Class (grams)	Adjusted NOAEL	EECs and RQs									
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Granivore	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
15	1.10	457.64	416.45	209.75	190.87	257.42	234.25	28.60	26.03	6.36	5.78
35	0.89	316.29	355.73	144.97	163.04	177.91	200.10	19.77	22.23	4.39	4.94
1000	0.38	73.33	190.68	33.61	87.40	41.25	107.26	4.58	11.92	1.02	2.65

Summary of Risk Quotient Calculations Based on Mean Kenega EECs

Table X. Mean Kenaga, Acute Avian Dose-Based Risk Quotients									
Size Class (grams)	Adjusted LD50	EECs and RQs							
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
20	0.75	193.80	259.200	82.08	109.779	102.60	137.224	15.96	21.346
100	0.95	110.50	116.091	46.80	49.168	58.50	61.460	9.10	9.560
1000	1.34	49.30	36.668	20.88	15.530	26.10	19.412	4.06	3.020

Table X. Mean Kenega, Subacute Avian Dietary Based Risk Quotients								
	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
LC50	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
32	170.00	5.313	72.00	2.250	90.00	2.813	14.00	0.438

Size class not used for dietary risk quotients

Table X. Mean Kenega, Chronic Avian Dietary Based Risk Quotients								
	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
NOAEC (ppm)	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
8	170.00	20.482	72.00	8.675	90.00	10.843	14.00	1.687

Size class not used for dietary risk quotients

Table X. Mean Kenaga, Acute Mammalian Dose-Based Risk Quotients											
Size Class (grams)	Adjusted LD50	EECs and RQs									
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Granivore	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
15	1109.90	161.50	0.146	68.40	0.062	85.50	0.077	13.30	0.012	2.94	0.00
35	898.03	112.20	0.125	47.52	0.053	59.40	0.066	9.24	0.010	2.10	0.00
1000	388.43	25.50	0.066	10.80	0.028	13.50	0.035	2.10	0.005	0.42	0.00

Table X. Mean Kenega, Acute Mammalian Dietary Based Risk Quotients								
LC50 (ppm)	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
0	170.00	#####	72.00	#####	90.00	#####	14.00	#####

Size class not used for dietary risk quotients

Table X. Mean Kenega, Chronic Mammalian Dietary Based Risk Quotients								
NOAEC (ppm)	EECs and RQs							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
10	170.00	17.000	72.00	7.200	90.00	9.000	14.00	1.400

Size class not used for dietary risk quotients

Table X. Mean Kenega, Chronic Mammalian Dose-Based Risk Quotients											
Size Class (grams)	Adjusted NOAEL	EECs and RQs									
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Granivore	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
15	1.10	161.50	146.963	68.40	62.243	85.50	77.804	13.30	12.103	2.94	2.68
35	0.89	112.20	126.189	47.52	53.445	59.40	66.806	9.24	10.392	2.10	2.36
1000	0.38	25.50	66.306	10.80	28.083	13.50	35.103	2.10	5.460	0.42	1.09

Appendix E. Output from T-HERPS v.1.0 model (single application to fig)

Summary of Risk Quotient Calculations Based on Upper Bound Kenaga EECs

Table X. Upper Bound Kenaga, Acute Terrestrial Herpetofauna Dose-Based Risk Quotients

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1.44	2.62	1.82	0.29	0.20	N/A	N/A	N/A	N/A	N/A	N/A
37	1.44	2.58	1.79	0.29	0.20	74.80	51.94	4.67	3.25	0.09	0.06
238	1.44	1.69	1.17	0.19	0.13	11.63	8.08	0.73	0.50	0.06	0.04

Table X. Upper Bound Kenaga, Subacute Terrestrial Herpetofauna Dietary Based Risk Quotients

LC50 (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
32	67.50	2.11	7.50	0.23	79.07	2.47	4.94	0.15	2.34	0.07

Size class not used for dietary risk quotients

Table X. Upper Bound Kenaga, Chronic Terrestrial Herpetofauna Dietary Based Risk Quotients

NOAEC (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
8	67.50	8.13	7.50	0.90	79.07	9.53	4.94	0.60	2.34	0.28

Size class not used for dietary risk quotients

Summary of Risk Quotient Calculations Based on Mean Kenaga EECs

Table X. Mean Kenaga, Acute Terrestrial Herpetofauna Dose-Based Risk Quotients

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1.44	0.87	0.61	0.14	0.094	N/A	N/A	N/A	N/A	N/A	N/A
37	1.44	0.86	0.60	0.13	0.093	26.49	18.397	2.18	1.52	0.03	0.02
238	1.44	0.56	0.39	0.09	0.061	4.12	2.860	0.34	0.24	0.02	0.01

Table X. Mean Kenaga, Subacute Terrestrial Herpetofauna Dietary Based Risk Quotients

LC50 (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
32	22.50	0.703	3.50	0.109	28.01	0.8752	2.31	0.072	0.78	0.024

Size class not used for dietary risk quotients

Table X. Mean Kenaga, Chronic Terrestrial Herpetofauna Dietary Based Risk Quotients

NOAEC (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
8	22.50	2.711	3.50	0.422	28.01	3.3741	2.31	0.278	0.78	0.094

Size class not used for dietary risk quotients

Appendix F. Sensitivity Distribution Data

Tables F.1-F.4 contain the 96-hour LC_{50} data for fish and associated calculations used to derive the species sensitivity distribution shown in Figure 21 (of risk assessment). Tables F.5-F.8 contain the 48 to 96-hour EC_{50} data for invertebrates and associated calculations used to derive the species sensitivity distribution shown in Figure 23 (of risk assessment). Tables F.9-F.12 contain LD_{50} data for birds and associated calculations used to derive the species sensitivity distribution shown in Figure 22 (of risk assessment).

Table F.1. Summary of 96 hour LC50 data for effects of diazinon on freshwater fish.

Common Name	Species Name	Mean LC50 (ppb)	Log 10 LC50	Test Subst. (% a.i.)	MRID/ Accession	ECOTOX Number	Comments
Bluegill sunfish	*Lepomis macrochirus*	136	2.134	91.0	104923	NA	cited in RED
Bluegill sunfish	*Lepomis macrochirus*	460	2.663	92.5	ROODI007	NA	cited in RED
Bluegill sunfish	*Lepomis macrochirus*	168	2.225	92.0	40094602	NA	cited in RED
Brook trout	*Salelinus fontinalis*	770	2.886	92.5	ROODI007	NA	cited in RED
Cutthroat trout	*Oncorhynchus clarki*	1700	3.230	92.0	40094602	NA	cited in RED
Fathead Minnow	*Pimephales promeals*	7800	3.892	92.5	ROODI007	NA	cited in RED
Flagfish	*Jordanella floridae*	1600	3.204	92.5	ROODI007	NA	cited in RED
Guppy	*Lebistes reticulatus*	1100	3.041	NR	5000811	NA	cited in RED
Lake trout	*Salevelinus namaychus*	602	2.780	92.0	40094602	NA	cited in RED
Rainbow trout	*Oncorhynchus gairdneri*	90	1.954	89.0	40094602	NA	cited in RED
Rainbow trout	*Oncorhynchus sp.*	400	2.602	91.0	104923	NA	cited in RED
NR = not reported, NA = not applicable							

Table F.2. Species Mean Acute Values (SMAVs) for freshwater fish.		
Common Name	**Species Name**	**Log10 SMAV**
Bluegill sunfish	*Lepomis macrochirus*	2.3405
Brook trout	*Salelinus fontinalis*	2.8865
Cutthroat trout	*Oncorhynchus clarki*	3.2304
Fathead Minnow	*Pimephales promeals*	3.8921
Flagfish	*Jordanella floridae*	3.2041
Guppy	*Lebistes reticulatus*	3.0414
Lake trout	*Salevelinus namaychus*	2.7796
Rainbow trout	*Oncorhynchus gairdneri*	1.954
Rainbow trout	*Oncorhynchus sp.*	2.602

Table F.3. Genus Mean Acute Values (GMAVs) for freshwater fish.					
Common Name	**Species Name**	**Log10 GMAV**	**GMAV LC50**	**Sensitivity Rank**	**Rank on curve**
sunfish	*Lepomis*	2.3405	219	1	0.00
Brook trout	*Salelinus*	2.8865	770	4	0.50
Trout	*Oncorhynchus*	2.5956	394	2	0.17
Fathead Minnow	*Pimephales*	3.8921	7800	7	1.00
Flagfish	*Jordanella*	3.2041	1600	6	0.83
Guppy	*Lebistes*	3.0414	1100	5	0.67
Lake trout	*Salevelinus*	2.7796	602	3	0.33
Genus Mean for All:		**2.9628**	**1784**		
Genus Standard Deviation for all:		**0.4982**	**2693**		

Table F.4. Calculation of species sensitivity distribution curve for freshwater fish.			
Proportion	**Z_P**	**Log10 point**	**Point Estimate**
0.05	-1.645	2.143229	139
0.10	-1.282	2.324089	211
0.20	-0.842	2.543314	349
0.25	-0.675	2.626761	423
0.30	-0.524	2.701754	503
0.40	-0.253	2.836776	687
0.50	0	2.962831	918
0.60	0.253	3.088885	1227
0.70	0.524	3.223907	1675
0.75	0.675	3.299141	1991
0.80	0.842	3.382347	2412
0.90	1.282	3.601572	3996
0.95	1.645	3.782432	6059

Z_P = (Log10 LC50 - fish mean GMAV)/(fish std GMAV)

Table F.5. Summary of 48-96 hour EC50 data for effects of diazinon on freshwater invertebrates.							
Common Name	**Species Name**	**Mean EC50 (ppb)**	**Log 10 EC50**	**Test Substance (% a.i.)**	**MRID**	**ECOTOX Number**	**Comments**
waterflea	*Ceriodaphnia dubia*	**0.21**	-0.678	**NA**		**76752**	
waterflea	*Ceriodaphnia dubia*	**0.45**	-0.347	**NA**		**76752**	
daphnid	*Simocephalus serrulatus*	1.34	0.127	89.0	40094602	NA	cited in RED, updated by 10-5-05 memo*
daphnid	*Simocephalus serrulatus*	1.67	0.223			NA	cited in 10-5-05 memo*
daphnid	*Daphnia pulex*	0.79	-0.102	89.0	40094602	NA	cited in RED, updated by 10-5-05 memo*
daphnid	*Daphnia magna*	0.83	-0.081	>89.0	109022	NA	cited in RED
mosquito larvae	*Culex pipiens fatigans*	35.0	1.544	NR	5000811	NA	cited in RED
scud	*Gammarus fasciatus*	2.0	0.299	89.0	40094602	NA	cited in RED, updated by 10-5-05 memo*
stonefly	*Pteronarcys californica*	20.49	1.312	89.0	40094602	NA	cited in RED, updated by 10-5-05 memo*

NR = not reported, NA = not applicable

Table F.6. Species Mean Acute Values (SMAVs) for freshwater invertebrates.		
Common Name	**Species Name**	**Log10 SMAV**
waterflea	*Ceriodaphnia dubia*	**-0.512**
daphnid	*Simocephalus sp.*	0.1749
daphnid	*Daphnia pulex*	-0.1024
daphnid	*Daphnia magna*	-0.0809
mosquito larvae	*Culex pipiens fatigans*	1.5441
scud	*Gammarus fasciatus*	0.2989
stonefly	*Pteronarcys sp.*	1.3115

Table F.7. Genus Mean Acute Values (GMAVs) for freshwater invertebrates.					
Common Name	**Species Name**	**Log10 GMAV**	**GMAV EC50**	**Sensitivity Rank**	**Rank on curve**
waterflea	*Ceriodaphnia dubia*	**-0.512**	0.31	1	0.00
daphnid	*Simocephalus*	0.1749	1.50	3	0.40
daphnid	*Daphnia*	-0.0916	0.81	2	0.20
mosquito larvae	*Culex*	1.5441	35.00	6	1.00
scud	*Gammarus*	0.2989	1.99	4	0.60
stonefly	*Pteronarcys*	1.3115	20.49	5	0.80
Genus Mean for All:		**0.4542**	**10**		
Genus Standard Deviation for all:		**0.8071**	**14**		

Table F.8. Calculation of species sensitivity distribution curve for freshwater invertebrates.			
Proportion	**Z_P**	**Log10 point**	**Point Estimate**
0.05	-1.645	-0.87343	0.13
0.10	-1.282	-0.58046	0.26
0.20	-0.842	-0.22533	0.60
0.25	-0.675	-0.09016	0.81
0.30	-0.524	0.031322	1.07
0.40	-0.253	0.250045	1.78
0.50	0	0.45424	2.85
0.60	0.253	0.658436	4.55
0.70	0.524	0.877159	7.54
0.75	0.675	0.99903	9.98
0.80	0.842	1.133816	13.61
0.90	1.282	1.488938	30.83
0.95	1.645	1.781914	60.52
Z_P = (Log10 LC50 - fish mean GMAV)/(fish std GMAV)			

Table F.9. Summary of acute oral toxicity of diazinon to birds.						
Common Name	**Species Name**	**Mean LD50 (mg a.i./kg)**	**Log 10 LD50**	**Test Substance (% a.i.)**	**MRID/ Accession**	**Comments**
Red-winged blackbird	*Agelaius phoeniceus*	3.2	0.505	>90.0	20560	cited in RED
Mallard Duck	*Anas platyrhynchos*	6.38	0.805	97.0	FEODIA06	cited in RED
Mallard Duck	*Anas platyrhynchos*	6.66	0.823	86.6	FEODIA04	cited in RED
Mallard Duck	*Anas platyrhynchos*	3.54	0.549	89.0	160000	cited in RED
Mallard Duck	*Anas platyrhynchos*	1.44	0.158	88.2	40895301	cited in RED
Mallard Duck	*Anas platyrhynchos*	14	1.146	86.6	Grimes and Jaber 1987	cited in RED
Mallard Duck	*Anas platyrhynchos*	8.7	0.940	89.2	FEODIA02	cited in RED
Mallard Duck	*Anas platyrhynchos*	3.16	0.500	86.6	FEODIA03	cited in RED. Value is actually <3.16.
Mallard Duck	*Anas platyrhynchos*	3.16	0.500	97.0	FEODIA05	cited in RED. Value is actually <3.16.
Mallard Duck	*Anas platyrhynchos*	6	0.778	86.6	FEODIA01	cited in RED. Value is actually >6 and <24.6.
Canada goose	*Branta canadensis*	6	0.778	86.6	FEODIA07	cited in RED. Value is actually >6.0 <39.3
Canada goose	*Branta canadensis*	6.16	0.790	86.6	FEODIA08	cited in RED
Northern bobwhite quail	*Colinus virginianus*	10	1.000	99.0	ROODI002	cited in RED
Northern bobwhite quail	*Colinus virginianus*	5.2	0.716	89.0	19015	cited in RED
brown headed cowbird	*Molothrus ater*	69	1.839	88.2	40895303	cited in RED
House sparrow	*Passer domesticus*	7.5	0.875	>90	20560	cited in RED
Ring-necked pheasant	*Phasianus colchicus*	4.33	0.636	89.0	160000	cited in RED

Table F.10. Species Mean Acute Values (SMAVs)		
Common Name	**Species Name**	**Log10 SMAV**
Red-winged blackbird	*Agelaius phoeniceus*	0.5051
Mallard Duck	*Anas platyrhunchos*	0.6888
Canada goose	*Branta canadensis*	0.7839
Northern bobwhite quail	*Colinus virginianus*	0.8580
brown headed cowbird	*Molothrus ater*	1.8388
House sparrow	*Passer domesticus*	0.8751
Ring-necked pheasant	*Phasianus colchicus*	0.636

Table F.11. Genus Mean Acute Values (GMAVs)					
Common Name	**Species Name**	**Log10 GMAV**	**GMAV LC50**	**Sensitivity Rank**	**Rank on curve**
Red-winged blackbird	*Agelaius*	0.5051	3	1	0.00
Mallard Duck	*Anas*	0.6888	5	3	0.33
Canada goose	*Branta*	0.7839	6	4	0.50
Northern bobwhite quail	*Colinus*	0.8580	7	5	0.67
brown headed cowbird	*Molothrus*	1.8388	69	7	1.00
House sparrow	*Passer*	0.8751	8	6	0.83
Ring-necked pheasant	*Phasianus*	0.6365	4	2	0.17
Genus Mean for All:		**0.8837**	**15**		
Genus Standard Deviation for all:		**0.4407**	**24**		

Table F.12. Calculation of species sensitivity distribution curve			
Proportion	**Z_P**	**Log10 point**	**Point Estimate**
0.05	-1.645	0.158748	1
0.10	-1.282	0.318731	2
0.20	-0.842	0.512649	3
0.25	-0.675	0.586464	4
0.30	-0.524	0.6528	4
0.40	-0.253	0.772236	6
0.50	0	0.883739	8
0.60	0.253	0.995243	10
0.70	0.524	1.114679	13
0.75	0.675	1.181228	15
0.80	0.842	1.254829	18
0.90	1.282	1.448748	28
0.95	1.645	1.608731	41
Z_P = (Log10 LC50 - fish mean GMAV)/(fish std GMAV)			

Appendix G. The Risk Quotient Method and Levels of Concern

The Risk Quotient Method is the means used by EFED to integrate the results of exposure and ecotoxicity data. For this method, Risk Quotients (RQs) are calculated by dividing exposure estimates by the acute and chronic ecotoxicity values (i.e., RQ = EXPOSURE/TOXICITY). These RQs are then compared to OPP's levels of concern (LOCs). These LOCs are criteria used by OPP to indicate potential risk to non-target organisms and the need to consider regulatory action. EFED has defined LOCs for acute risk, potential restricted use classification, and for endangered species.

The criteria indicate that a pesticide used as directed has the potential to cause adverse effects on non-target organisms. LOCs currently address the following risk presumption categories:

(1) acute - there is a potential for acute risk; regulatory action may be warranted in addition to restricted use classification;

(2) acute restricted use - the potential for acute risk is high, but this may be mitigated through restricted use classification;

(3) acute endangered species - the potential for acute risk to endangered species is high, regulatory action may be warranted; and

(4) chronic risk - the potential for chronic risk is high, regulatory action may be warranted.

Currently, EFED does not perform assessments for chronic risk to plants, acute or chronic risks to non-target insects, or chronic risk from granular/bait formulations to mammalian or avian species.

The ecotoxicity test values (i.e., measurement endpoints) used in the acute and chronic RQs are derived from required studies. Examples of ecotoxicity values derived from short-term laboratory studies that assess acute effects are: (1) LC50 (fish and birds), (2) LD50 (birds and mammals), (3) EC50 (aquatic plants and aquatic invertebrates), and (4) EC25 (terrestrial plants). Examples of toxicity test effect levels derived from the results of long-term laboratory studies that assess chronic effects are: (1) the Lowest Observed Adverse Effect Concentration (LOAEC) (birds, fish, and aquatic invertebrates), and (2) the No Observed Adverse Effect Concentration (NOAEC) (birds, fish and aquatic invertebrates). The NOAEC is generally used as the ecotoxicity test value in assessing chronic effects. Risk presumptions, along with the corresponding RQs and LOCs are summarized in Table G-1.

Table G-1. Agency risk quotient (RQ) metrics and levels of concern (LOC) per risk class.			
Risk Class	**Risk Description**	**RQ**	**LOC**
Aquatic Animals (fish and invertebrates)			
Acute	Potential for effects to non-listed animals from acute exposures	Peak EEC/LC_{50}[1]	0.5
Acute Restricted Use	Potential for effects to animals from acute exposures Risks may be mitigated through restricted use classification	Peak EEC/LC_{50}[1]	0.1
Acute Listed Species	Listed species may be potentially affected by acute exposures	Peak EEC/LC_{50}[1]	0.05
Chronic	Potential for effects to non-listed and listed animals from chronic exposures	60-day EEC/NOEC (fish) 21-day EEC/NOEC (invertebrates)	1
Terrestrial Animals (mammals and birds)			
Acute	Potential for effects to non-listed animals from acute exposures	EEC[2]/LC_{50} (Dietary) EEC/LD_{50} (Dose)	0.5
Acute Restricted Use	Potential for effects to animals from acute exposures Risks may be mitigated through restricted use classification	EEC[2]/LC_{50} (Dietary) EEC/LD_{50} (Dose)	0.2
Acute Listed Species	Listed species may be potentially affected by acute exposures	EEC [2]/LC_{50} (Dietary) EEC/LD_{50} (Dose)	0.1
Chronic	Potential for effects to non-listed and listed animals from chronic exposures	EEC [2]/NOAEC	1
Plants			
Non-Listed	Potential for effects to non-target, non-listed plants from exposures	EEC/ EC_{25}	1
Listed Plant	Potential for effects to non-target, listed plants from exposures	EEC/ NOEC EEC/ EC_{05}	1

[1] LC_{50} or EC_{50}. [2] Based on upper bound Kenaga values.

Appendix H. List of citations accepted and rejected by ECOTOX criteria

The citations in this appendix were accepted by ECOTOX. Citations include the ECOTOX Reference number. References in section H.1 those relevant to diazinon which were cited within this risk assessment. References in section H.2 were those relevant to diazinon which were not cited within the risk assessment. References in section H.3 those relevant to degredates of diazinon which were cited within this risk assessment. References in section H.4 were those relevant to degredates of diazinon which were not cited within the risk assessment. In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

- the toxic effects are related to single chemical exposure;
- the toxic effects are on an aquatic or terrestrial plant or animal species;
- there is a biological effect on live, whole organisms;
- a concurrent environmental chemical concentration/dose or application rate is reported; and
- there is an explicit duration of exposure.

Section H.5 includes the list of exclusion terms and descriptions for citations not accepted by ECOTOX. For diazinon, there were hundreds of references that were not accepted by ECOTOX for one or more of the reasons included in section H.5. A full list of the citations reviewed and rejected by the criteria for ECOTOX is listed in section H.6.

H.1. ECOTOX accepted references, relevant to diazinon, cited within the risk assessment or used for deriving species sensitivity distributions

664 Allison DT;Hermanutz RO; (1977) Toxicity of Diazinon to Brook Trout and Fathead Minnows. (): 69 p.(Author Communication Used)-.

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885 Sanders HO; (1969) Toxicity of Pesticides to the Crustacean Gammarus lacustris. (): 18 p. (Author Communication Used)(Used with Reference 732) (Publ in Part As 6797)-.

4009 Fernandez-Casalderrey A;Ferrando MD;Andreu-Moliner E; (1994) Effect of Sublethal Concentrations of Pesticides on the Feeding Behavior of Daphnia magna. 27(1): 82-89.

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6449 Dortland RJ; (1980) Toxicological Evaluation of Parathion and Azinphosmethyl in Freshwater Model Ecosystems. 898(): 1-112 (Author Communication Used).

6728 Sancho E;Ferrando MD;Gamon M;Andreu-Moliner E; (1992) Organophosphorus Diazinon Induced Toxicity in the Fish Anguilla anguilla L. 103(2): 351-356.

7004 Sancho E;Ferrando MD;Andreu E;Gamon M; (1993) Bioconcentration and Excretion of Diazinon by Eel.

11055 Ferrando MD;Sancho E;Andreu-Moliner E; (1991) Comparative Acute Toxicities of Selected Pesticides to Anguilla anguilla. B26(5/6): 491-498.

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15687 Sancho E;Ferrando MD;Gamon M;Andreu-Moliner E; (1994) Uptake and Clearance of Diazinon in Different Tissues of the European Eel (Anguilla anguilla L.). 7(1): 41-49.

16043 Norberg-King TJ; (1987) "Toxicity Data on Diazinon, Aniline, 2,4-Dimethylphenol". (): -.

16547 Oh HS;Lee SK;Kim YH;Roh JK; (1991) Mechanism of Selective Toxicity of Diazinon to Killifish (Oryzias latipes) and Loach (Misgurnus anguillicaudatus). (): 343-353.

16844 Bailey HC;DiGiorgio C;Kroll K;Miller JL;Hinton DE;Starrett G; (1996) "Development of Procedures for Identifying Pesticide Toxicity in Ambient Waters: Carbofuran, Diazinon, Chlorpyrifos". 15(6): 837-845.

18129 Werner I;Nagel R; (1997) "Stress Proteins HSP60 and HSP70 in Three Species of Amphipods Exposed to Cadmium, Diazinon, Dieldrin and Fluoranthene". 16(11): 2393-2403.

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887 Sanders HO; (1972) Toxicity of Some Insecticides to Four Species of Malacostracan Crustaceans. (): 19 p. (Publ in Part As 6797)-.

2093 Naqvi SM;Ferguson DE; (1968) Pesticide Tolerances of Selected Freshwater Invertebrates. 14(): 121-127.

2155 Henderson C;Pickering QH; (1958) Toxicity of Organic Phosphorus Insecticides to Fish. 87(): 39-51.

2400 Davis HC;Hidu H; (1969) Effects of Pesticides on Embryonic Development of Clams and Oysters and on Survival and Growth of the Larvae. 67(2): 393-404.

2904 Hilsenhoff WL; (1959) The Evaluation of Insecticides for the Control of Tendipes plumosus (Linnaeus).

5311 Dennis WH Jr.;Rosencrance AB;Randall WF; (1980) Acid Hydrolysis of Military Standard Formulations of Diazinon. 15(1): 47-60.

6797 Mayer FL Jr.;Ellersieck MR; (1986) Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals. (): 505 p. (USGS Data File)-.

8039 Ukeles R; (1962) Growth of Pure Cultures of Marine Phytoplankton in the Presence of Toxicants. 10():

14530 Batte EG;Swanson LE; (1952) "Laboratory Evaluation of Organic Compounds as Molluscacides and Ovocides, II". 38(): 65-68.

18398 Tsuda T;Kojima M;Harada H;Nakajima A;Aoki S; (1997) "Acute Toxicity, Accumulation and Excretion of Organophosphorous Insecticides and Their Oxidation Products in Killifish". 35(5): 939-949.

37780 Lynch WT;Coon JM; (1972) Effect of Tri-o-Tolyl Phosphate Pretreatment on the Toxicity and Metabolism of Parathion and Paraoxon in Mice. 21(): 153-165.

H.5. List of exclusion terms utilized for reviewing studies considered for ECOTOX database

Review--all toxicity tests reported elsewhere. If the publication is applicable to one of the ECOTOX databases, the bibliography is skimmed and any applicable articles are ordered.

Methods--no usable toxicity tests. Reports of methods of conducting tests, determination or purification of chemicals, etc. Methods publications are selected to be ordered for the ECOTOX toxicology methods information file (Methfile).

Modeling only, no new organism exposure data. Modeling studies may report original toxicity tests performed as comparisons or as a basis for extrapolation; order the paper if it is not clear from the abstract.

Other ambient conditions--effects on organisms from changes in conditions other than addition of chemicals, including radioactivity, ultraviolet light (UV), temperature, pH, salinity, dissolved oxygen (DO), or other water, air, or soil parameters.

Biological Toxicant--includes venoms, fungal toxins, *Bacillus thuringiensis*, other plant, animal, or microbial extracts or toxins.

Drug--testing for drug effects and side-effects .

Effluent, sewage, or polluted runoff.

Mixture--no single chemical tests reported.

Nutrient studies--in situ chemicals tested as nutrients.

No Species--no organism present or tested or unable to verify a species or exposure of dead organism.

In Vitro studies, including exposure of cell cultures and excised tissues.

Bacteria as test organism, including **Microtox** tests, or other microbial organisms.

Yeast as a test organism is historically not coded in ECOTOX.

No Toxicity **Data**--publications which are not toxicology studies.

Human Health effects; studies with human subjects or with animal subjects as surrogates for human health risk assessment.

No Concentration--no usable dose or concentration reported; identified after examination of full paper. Includes lead-shot studies which lack dose information or give only number of pellets. Concentrations reported only in log units are not coded.

Sediment Concentration--chemical concentration reported in sediment only. Sediment studies are coded for AQUIRE only if a water concentration of the added chemical is also reported; order the publication if unclear from the abstract.

No Duration reported, identified after examination of full paper.

Incident papers--reports of animal deaths by poison, etc. Lacks usable concentration or duration or both.

Survey studies--measuring amounts of chemical present, but no usable quantification of exposure. Lacks either usable concentration or duration or both.

Fate: Studies reporting only what happens to the chemical in abiotic matrices

Food Studies, no chemical and effects information are reported

PUBL AS, author has results were published in a different format. For example, may be used for a Ph.D. dissertation when the same results were also published in a peer-reviewed journal.

NON-ENGLISH or **FORE**, paper was published in a foreign language.

H.6. List of diazinon related studies excluded from ECOTOX database

1. 2000). Farm Chemicals Handbook. *Meister Publishing Company, Willoughby, Ohio* 4 p.
   Rejection Code: REVIEW.

2. 1989). Identification and characterization of a plasma protein group, the thiol ester plasma proteins (TEPP) which bind IL-1[beta] : +NY Hosp.Cornell Univ., NY 10021; *Dept.Derm.II and LBI-DVS, Lab.Cellbiol., Univ.Vienna; #Dept.Mol.Biol., Univ.Aarhus, Denmark. *Cytokine* 1: 89.
   Rejection Code: METHODS.

3. 1991). INITIAL SUBMISSION: ACUTE SKIN ABSORPTION TEST ON RABBITS - ALD (FINAL REPORT) WITH COVER LETTER DATED 112691. *EPA/OTS; Doc #88-920000406*.
   Rejection Code: HUMAN HEALTH.

4. 1992). INITIAL SUBMISSION: GUINEA PIG SENSITIZATION (FINAL REPORT) WITH COVER LETTER DATED 010792. *EPA/OTS; Doc #88-920000599*.
   Rejection Code: HUMAN HEALTH.

5. 1991). INITIAL SUBMISSION: SKIN ABSORPTION ALD - RABBITS (FINAL REPORT) WITH COVER LETTER DATED 112691. *EPA/OTS; Doc #88-920000404*.
   Rejection Code: HUMAN HEALTH.

6. 2000). LIST OF THE ORGANIC MICROPOLLUTANTS FOUND IN SOME FRESH WATERS, EFFLUENTS, AQUATIC PLANTS AND ANIMALS, AND BOTTOM SEDIMENTS. *EPA/OTS; Doc #40-7642395*.
   Rejection Code: NO TOX DATA.

7. 1991). Puget Sound pesticide reconnaissance survey, 1990.
   Rejection Code: SURVEY.

8. 2000). SUPPORT DOCUMENT FOR THE SARA SECTION 110 "SECOND 100" LIST (DRAFT). *EPA/OTS; Doc #110-881013* .
   Rejection Code: NO TOX DATA.

9. 1979). UPDATE ON PROGRESS OF INFORMATION RELATING TO CHEMICAL FERTILIZER PLANT WITH ATTACHMENTS. *EPA/OTS; Doc #FYI-OTS-1278-0017*.
   Rejection Code: NO TOX DATA.

10. ABD-EL SAMEI HA, HANDY RD, BAYOMY MFF, MAHRAN HA, ABDEEN AM, and EL-ELAIMY EA (1999). Histopathological effects of chronic diazinon exposure and dietary modulation on selected haematopoietic tissues of the mouse. *ANNUAL CONGRESS OF THE BRITISH TOXICOLOGY SOCIETY, STOKE ON TRENT, ENGLAND, UK, APRIL 18-21, 1999.YHUMAN & EXPERIMENTAL TOXICOLOGY; 18* 533.
    Rejection Code: ABSTRACT.

11. ABDALLA, A. (1992). SUDAN THE SITUATION OF DESERT LOCUST TREE LOCUST AND GRASSHOPPERS IN THE SUDAN 1990-1991. *LOMER, C. J. AND C. PRIOR (ED.). BIOLOGICAL CONTROL OF LOCUSTS AND GRASSHOPPERS; WORKSHOP, COTONOU, BENIN, APRIL 29-MAY 1, 1991. XII+394P. C.A.B. INTERNATIONAL: WALLINGFORD, ENGLAND, UK; INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE: IBADAN, NIGERIA. ISBN 0-85198-779-6.; 0 (0). 1992. 97-98.*
    Rejection Code: INCIDENT.

12. Abdel-Halim, K. Y., Salama, A. K., El-khateeb, E. N., and Bakry, N. M. (Organophosphorus pollutants

(OPP) in aquatic environment at Damietta Governorate, Egypt: Implications for monitoring and biomarker responses. *Chemosphere* In Press, Corrected Proof.
Rejection Code: SURVEY.

13. Abdelsalam, E. B. (1999). Neurotoxic Potential of Six Organophosphorus Compounds in Adult Hens. *Vet.Hum.Toxicol.* 41: 290-292.

; Habitat: T; Effect Codes: BCM,PHY

14. Abdelsalam, E. B. and Ford, E. J. H. (1987). The effect of induced liver, kidney and lung lesions on the toxicity of levamisole and diazinon in calves. *Journal of Comparative Pathology* 97: 619-627.
Rejection Code: MIXTURE.

15. Abdelsalam, E. B. and Ford, E. J. H. (1986). Effect of pretreatment with hepatic microsomal enzyme inducers on the toxicity of diazinon in calves. *Research in Veterinary Science [RES. VET. SCI.]. Vol. 41, no. 3, pp. 336-339. 1986.*
Rejection Code: MIXTURE.

16. Abou-Arab, A. A. K. and Abou Donia, M. A. (2001). Pesticide residues in some Egyptian spices and medicinal plants as affected by processing. *Food Chemistry* 72: 439-445.
Rejection Code: FATE.

17. ABOU-ARAB A AK (1999). Behavior of pesticides in tomatoes during commercial and home preparation.
Rejection Code: FATE.

18. ABOU-ARAB AAK, KAWTHER, M. SOLIMAN, EL TANTAWY ME, BADEAA, R. ISMAIL, and KHAYRIA, N. (1999). Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *FOOD CHEMISTRY; 67* 357-363.
Rejection Code: HUMAN HEALTH.

19. Abu-Qare, A. W. and Abou-Donia, M. B. (2001). Inhibition and Recovery of Maternal and Fetal Cholinesterase Enzyme Activity Following a Single Cutaneous Dose of Methyl Parathion and Diazinon, Alone and in Combination, in Pregnant Rats. *J.Appl.Toxicol.* 21: 307-316.

; Habitat: T; Effect Codes: BCM

20. Adachi, K., Ohokuni, N., and Mitsuhashi, T. (1984). Simple analytical method for organophosphorus pesticide determination in unpolished rice, using removal of fats by zinc acetate. *Journal of the Association of Official Analytical Chemists [J. ASSOC. OFF. ANAL. CHEM.]. Vol. 67, no. 4, pp. 798-800. 1984.*
Rejection Code: METHODS.

21. Adams, P. B. and Wong, J. A. L. (1991). The Effect of Chemical Pesticides on the Infection of Sclerotia of Sclerotinia minor by the Biocontrol Agent Sporidesmium sclerotivorum. *Phytopathology* 81: 1340-1343.

; Habitat: T; Effect Codes: PHY

22. Adlung, K. G. (1957). The Toxicity of Insecticidal and Acaricidal Agents to Fish (Zur Toxizitat Insektizider und Akarizider Wirkstoffe fur Fische). *Naturwissenschaften* 44: 471-472.

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23. Adonaylo, V. N. and Oteiza, P. I. (1999). Pb2+ promotes lipid oxidation and alterations in membrane physical properties. *Toxicology* 132: 19-32.

Rejection Code: NO TOX DATA.

24. Ageda, Saori , Fuke, Chiaki, Ihama, Yoko, and Miyazaki, Tetsuji (The stability of organophosphorus insecticides in fresh blood. *Legal Medicine* In Press, Corrected Proof.
Rejection Code: FATE.

25. AHMAD, M. (1990). The effect of PB and DEF synergists on the toxicities of endosulfan, diazinon and monocrotophos in Heliothis armigera. *J PLANT PROT TROP; 7* 117-122.
Rejection Code: MIXTURE.

26. Ahmad, M. H. and Vekataraman, G. S. (1973). Tolerance of Aulosira fertilissima to Pesticides. *Curr.Sci.* 42: 108 (ABS).
Rejection Code: ABSTRACT.

27. Akasu, T. and Karczmar, A. G. (1980). Effects of anticholinesterases and of sodium fluoride on neuromyal desensitization. *Neuropharmacology* 19: 393-403.
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28. AKEY WC, RUSSELL, T., ALFORD, C., MORRISON, T., and DENNING, M. (1997). Will the toad croak? An endangered species decision case. *JOURNAL OF NATURAL RESOURCES AND LIFE SCIENCES EDUCATION; 26* 148-156.
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Rejection Code: FATE.

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Rejection Code: IN VITRO.

33. AL-SAMARIEE AI, SHAKER, K. AM, and AL-BASSOMY MA (1988). RESIDUE LEVELS OF THREE ORGANOPHOSPHORUS INSECTICIDES IN SWEET PEPPER GROWN IN COMMERCIAL GREENHOUSES. *PESTIC SCI; 22* 189-194.
Rejection Code: FATE.

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; Habitat: A; Effect Codes: MOR

35. ALAEE, M., FANG, X., YOUNG, S., TODD, A., STRUGER, J., HARRIS, M., and BESTARI, K. (1998). THE ANALYSIS OF ORGANOPHOSPHOROUS PESTICIDES IN SURFACE WATER USING SOLID-PHASE DISK EXTRACTION AND GC-MS. *INTERNATIONAL ASSOCIATION FOR GREAT LAKES RESEARCH. 41ST CONFERENCE OF THE INTERNATIONAL ASSOCIATION*

FOR GREAT LAKES RESEARCH; MEETING, HAMILTON, ONTARIO, CANADA, MAY 18-22, 1998. 160P. INTERNATIONAL ASSOCIATION FOR GREAT LAKES RESEARCH: ANN ARBOR, MICHIGAN, USA.; 0 137.
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; Habitat: A; Effect Codes: MOR

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Rejection Code: SURVEY.

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39. Albert, A. (1981). Selective Toxicity: The Physio-chemical Basis of Therapy. *6th Edition, The Chaucer Press Ltd., Bungay, Suffolk, England*.
Rejection Code: NO TOX DATA.

40. Alexander, J. P. (1983). Probable diazinon poisoning in peafowl: A clinical description. *Veterinary Record [VET. REC.]. Vol. 113, no. 19-20, 470 p. 1983.*
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41. Ali, Mezher A., Hough, Leslie, and Richardson, Anthony C. (1992). Thio and epidithio derivatives of methyl [beta]-lactoside. *Carbohydrate Research* 216: 271-287.
Rejection Code: METHODS.

42. ALI, S., HAQ, R., KHALIQ, M., and SHAKOORI AR (1997). Use of ultra-violet spectrophotometry for determination of insecticides and aromatic hydrocarbon pollutants. *PUNJAB UNIVERSITY JOURNAL OF ZOOLOGY; 12* 31-34.
Rejection Code: METHODS.

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Rejection Code: METHODS.

44. Allender, W. J. and Britt, A. G. (1994). Analyses of Liquid Diazinon Formulations and Breakdown Products: An Australia-Wide Survey. *Bull.Environ.Contam.Toxicol.* 53: 902-906.
Rejection Code: NO TOX DATA/SURVEY.

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; Habitat: A; Effect Codes: GRO,MOR,REP

46. Altuntas, I. , Kilinc, I., Orhan, H., Demirel, R., Koylu, H., and Delibas, N. (2004). The effects of diazinon on lipid peroxidation and antioxidant enzymes in erythrocytes in vitro. *Human & Experimental Toxicology [Hum. Exp. Toxicol.]. Vol. 23, no. 1, pp. 9-13. Jan 2004.*
Rejection Code: IN VITRO.

47. Amato, J. R., Mount, D. I., Durhan, E. J., Lukasewycz, M. T., Ankley, G. T., and Robert, E. D. (1992). An Example of the Identification of Diazinon as a Primary Toxicant in an Effluent. *Environ.Toxicol.Chem.* 11: 209-216.
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; Habitat: A; Effect Codes: BEH

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Rejection Code: SURVEY.

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51. Anderson, B. S., Hunt, J. W., Phillips, B. M., Nicely, P. A., Gilbert, K. D., DeVlaming, V., Connor, V., Richard, N., and Tjeerdema, R. S. (2003). Ecotoxicologic impacts of agricultural drain water in the Salinas River, California, USA. *Environmental Toxicology and Chemistry [Environ. Toxicol. Chem.]. Vol. 22, no. 10, pp. 2375-2384. Oct 2003.*
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; Habitat: A; Effect Codes: BCM

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; Habitat: A; Effect Codes: CEL

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; Habitat: A; Effect Codes: BEH,MOR

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; Habitat: A; Effect Codes: MOR

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Rejection Code: FATE.

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Rejection Code: NO SPECIES.

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Rejection Code: METHODS.

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Rejection Code: METHODS.

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Appendix I. Individual Effect Analysis

As discussed in the effects assessment section of the chapter, OPP conducted an analysis of U.S.G.S. data used to support the Mayer and Ellerseick data set. The analysis included 48-hr acute toxicity data for freshwater aquatic invertebrates including *Simocephalus serrulatus*, *Daphnia pulex*, *Gammarus fasciatus* and *Pteronarcys californica* (**Table I1**). Across the four species, the 48-hr probit dose response slope ranged from 5.74 to 6.90; the mean slope and standard error of the mean were 6.34 and 0.21, respectively. Since a probit dose-response slope is not available for the most the most sensitive species, *i.e.*, *Ceriodaphnia dubia*, the mean slope of 6.34 will be used in the analysis of potential individual effects discussed below.

Table I1. Acute 48-hr and 96-hr LC_{50} values for freshwater aquatic invertebrates based on USGS data used in support of Mayer and Ellerseick.

Species	48-hr LC_{50} (95% CI)	Slope	96-hr LC_{50}	Slope
Simocephalus serrulatus	1.34 (1.00 – 1.71)	6.9	no data	--
S. serrulatus	1.67 (1.31 – 2.16)	6.71	no data	--
Daphnia pulex	0.79 (0.58 – 1.02)	6.20	no data	--
Gammarus fasciatus	4.71 (3.69 – 6.11)	6.13	1.99 (1.48 – 2.63)	4.67
Pteronarcys califonica	59.4 (42.5 – 83.3)	5.74	20.5	22.7

Likelihood of individual acute effects to freshwater vertebrates based on maximum RQ value of 0.66 (2 aerial applications to lettuce) is 1 in 5 (**Figure I1**). At the acute risk to endangered species LOC of 0.05, the likelihood of individual effects 1 in 4.2 x10^8 (**Figure I2**)

IEC V1 - Individual Effect Chance Model Version 1
Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	90	Note: This is <u>not</u> used in calculation, just serves as a reminder to user
Enter desired threshold	0.66	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	4.5	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	-0.81205229	z is the standard normal deviate
Probability associated with z	0.20838081	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	4.80E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50}+(z/b)$
where: z is the standard normal deviate and b equals slope
Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)
Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I1. Estimation of likelihood on individual mortality based on risk quotients for freshwater vertebrates (RQ=0.66) following 2 applications per year to lettuce. Estimated dose-response slope is 4.5.

IEC V1 - Individual Effect Chance Model Version 1

Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	90	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	0.05	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	4.5	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	-5.85463498	z is the standard normal deviate
Probability associated with z	2.3903E-09	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	4.18E+08	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$

where: z is the standard normal deviate and b equals slope

Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)

Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I2. Estimation of likelihood on individual mortality based on risk quotients for freshwater vertebrates equivalent to the endangered species LOC (RQ=0.05) . Estimated dose-response slope is 4.5.

Likelihood of individual acute mortality in freshwater invertebrates based on and RQ value of 283 following 2 applications to lettuce is 100% (**Figure I3**). Even at RQ values of 3, the likelihood of individual mortality is 100% (**Figure I4**).

IEC V1 - Individual Effect Chance Model Version 1

Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	0.21	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	283	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	6.34	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	15.544326	z is the standard normal deviate
Probability associated with z	1	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	1.00E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$

where: z is the standard normal deviate and b equals slope

Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)

Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I3. Estimation of likelihood of individual mortality based on risk quotients for freshwater invertebrates (RQ=283) following 2 applications per year to lettuce. Estimated dose-response slope is 6.34.

IEC V1 - Individual Effect Chance Model Version 1
Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	0.21	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	3	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	6.34	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	3.02494875	z is the standard normal deviate
Probability associated with z	0.99875662	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	1.00E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$
where: z is the standard normal deviate and b equals slope
Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)
Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I4. Estimation of likelihood of individual mortality based on risk quotients for freshwater invertebrates (RQ=3). Estimated dose-response slope is 6.34.

The likelihood of mortality in terrestrial-phase CRLF based on dose-based RQ value of 1.82 for 1.4-g frogs feeding on small insects (RQ=1.82) is roughly 100% (**Figure I5**). At RQ values at the acute risk to endangered species LOC (RQ=0.1), the likelihood of individual mortality is 1 in 570 (**Figure I6**).

IEC V1 - Individual Effect Chance Model Version 1
Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	1.44	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	1.82	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	2.92	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	0.75940845	z is the standard normal deviate
Probability associated with z	0.77619587	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	1.29E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$
where: z is the standard normal deviate and b equals slope
Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)
Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I5. Estimation of likelihood of individual mortality based on dose-based risk quotients for terrestrial-phase CRLF (RQ=1.82) resulting from applications to figs. Estimated dose-response slope is 2.92.

IEC V1 - Individual Effect Chance Model Version 1		
Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate		
Enter LC_{50} or LD_{50}	1.44	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	0.1	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	2.92	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	-2.92	z is the standard normal deviate
Probability associated with z	0.00175016	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	5.71E+02	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$
where: z is the standard normal deviate and b equals slope
Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)
Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

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Figure I6. Estimation of likelihood of individual mortality at the acute risk to endangered species LOC of 0.1. Estimated dose-response slope is 2.92.

The likelihood of individual mortality in terrestrial-phase CRLF using a dietary-based RQ value for frogs feeding on small insects (RQ=2.11) is roughly 100% (**Figure I7**). However, with a subacute dietary dose-response slope of 5.6, the likelihood of individual effects at the endangered species LOC is 1 in 9.3 x 10^7 (**Figure I8**).

IEC V1 - Individual Effect Chance Model Version 1		
Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate		
Enter LC_{50} or LD_{50}	32	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	2.11	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	5.6	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	1.81598175	z is the standard normal deviate
Probability associated with z	0.96531341	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	1.04E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$
where: z is the standard normal deviate and b equals slope
Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)
Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

Ed Odenkirchen, May 28, 2003 EFED/OPP/USEPA

Figure I7. Estimation of likelihood of individual mortality using a dietary-based risk quotients for terrestrial-phase CRLF (RQ=2.11) resulting from diazinon applications to figs. Estimated dose-response slope is 5.6.

IEC V1 - Individual Effect Chance Model Version 1

Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}		32	Note: This is <u>not</u> used in calculation, just serves as a reminder to user
Enter desired threshold		0.1	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response		5.6	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result		-5.6	z is the standard normal deviate
Probability associated with z		1.0718E-08	Uses Excel NORMDIST function to estimate P
Chance of individual effect,	~1 in . . .	9.33E+07	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50}+(z/b)$

where: z is the standard normal deviate and b equals slope

Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)

Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

Ed Odenkirchen, May 28, 2003 EFED/OPP/USEPA

Figure I8. Estimation of likelihood of individual mortality at the acute risk to endangered species LOC of 0.1. Estimated subacute dietary dose-response slope is 5.6.

Likelihood of individual mortality to amphibian prey items using a dietary-based RQ value of 17 and a default dose-response slope of 5.6 is 100% (**Figure I9**). At the highest RQ values for mammalian prey items (RQ=0.47) the likelihood of individual mortality of mammalian prey is 1 in 14 (<10%) (**Figure I10**)

IEC V1 - Individual Effect Chance Model Version 1

Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}		32	Note: This is <u>not</u> used in calculation, just serves as a reminder to user
Enter desired threshold		17	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response		5.6	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result		6.89051396	z is the standard normal deviate
Probability associated with z		1	Uses Excel NORMDIST function to estimate P
Chance of individual effect,	~1 in . . .	1.00E+00	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50}+(z/b)$

where: z is the standard normal deviate and b equals slope

Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)

Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

Ed Odenkirchen, May 28, 2003 EFED/OPP/USEPA

Figure I9. Estimation of likelihood of individual mortality of amphibian prey items (RQ=17) and a dose-response slope is 5.6.

IEC V1 - Individual Effect Chance Model Version 1

Predictor of chance of individual effect using probit dose-response curve slope and median lethal estimate

Enter LC_{50} or LD_{50}	882	Note: This is not used in calculation, just serves as a reminder to user
Enter desired threshold	0.47	Note: This is either the RQ fraction of the toxicity endpoint, the EEC or dose fraction of the dose/concentration at tox endpoint, or the LOC
Enter slope of dose-response	4.5	Note: This is the slope of the dose response relationship from the study providing the above endpoint
z score result	-1.47555964	z is the standard normal deviate
Probability associated with z	0.07003107	Uses Excel NORMDIST function to estimate P
Chance of individual effect, ~1 in . . .	1.43E+01	Calculated as 1/P rounded to 0 decimals

This is based on the formula $\log LC_k = \log LC_{50} + (z/b)$

where: z is the standard normal deviate and b equals slope

Works for dose-response models based on a probit assumption (i.e. log normal distribution of individual sensitivity)

Note: Probability asociated with z value may be reported as "0". This is due to the inability of Excel to handle extremes in z scores beyond -8.2
In such cases the chance of individual effect is defaulted to 1 in 10^{16}, which is the limit of Excel reporting.

Ed Odenkirchen, May 28, 2003 EFED/OPP/USEPA

Figure I10. Estimation of likelihood of individual mortality of mammalian prey items (RQ=0.36) and a default dose-response slope is 4.5.

Appendix J. Use closure memo from SRRD concerning diazinon uses

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

June 18, 2007

MEMORANDUM

SUBJECT: **SRRD and RD Response to EFED Regarding the Current Label and Use Information for Diazinon (057801) to Support an Endangered Species Effects Determination.**

FROM: Jude Andreasen, Chemical Review Manager Jude Andreasen
Reregistration Branch I, Special Review and Reregistration Division

TO: Thomas Steeger, PhD., Senior Biologist
Kristina Garber, Biologist
Environmental Risk Branch 4, Environmental Fate and Effects Division

CC: Marion Johnson, Chief
Dana Pilitt, Product Manager
Insecticide Branch, Registration Branch

Steve Jarboe, Team Leader
Biological and Economic Analysis Division

THRU: Susan Lewis, Chief Susan Lewis
Laura Parsons, Team Leader Laura Parsons
Reregistration Branch I, Special Review and Reregistration Division

In support of the Environmental Fate and Effects Division's (EFED's) endangered species assessment of the California red-legged frog (CRLF), the Special Review and Reregistration Division (SRRD) and the Registration Division (RD) have reviewed the current diazinon labels and prepared the following comments.

SRRD obtained the most recently stamped diazinon labels from Dana Pilitt of the Registration Division (RD) to verify if RD has made any recent label changes on existing registrations of diazinon. Dana Pilitt confirmed that RD does not have pending actions to increase rates for diazinon higher than those listed on the current labels.

There are 38 active diazinon products, 21 of which are Special Local Need (SLN) registrations, including 3 for California, and 3 of which are manufacturing use products (MUPs). The LUIS Report included 37 products, because an additional SLN was added for Oregon in June 2007. Table 1 below provides a complete list of the 17 registered diazinon products as of June 15, 2007, excluding MUPs and SLNs outside of California.

Table 1. Complete List of Registered Diazinon Products (as of 06/15/07) Excluding MUPs and SLNs Outside of CA		
Registration Number	**More recent label than on LUIS Report**	**Comment**
2935-408	Yes 05/02/07	Voluntary cancellation, no additional uses or higher rates; use sites reduced to one crop
4581-392	Yes 12/28/06	No additional uses or higher rates
5905-248	No	
19713-91	No	
19713-492	Yes 12/07/06	No additional uses or higher rates
66222-9	No	
66222-10	No	
66222-103	No	
CA000030	No	
CA030014	No	
CA050002	No	Quarantine: to be used as needed by authorized government personnel.
011556-00123	No	Cattle ear tag
039039-00003	No	Cattle ear tag
039039-00006	No	Cattle ear tag
061483-00078	No	Cattle ear tag
061483-00080	No	Cattle ear tag
061483-00092	No	Cattle ear tag

SRRD verified the information presented in the table in EFED's memorandum dated June 4, 2007, and found several discrepancies with a label reference. The EFED table (reproduced below) lists sweet potatoes, but we did not find sweet potatoes on any diazinon label. We did find ginseng and filberts (hazelnuts) on several labels at the maximum rate of 0.5 lb. per year, which did not appear on the EFED table.

We received a voluntary cancellation for one of the labels listed, # 2935 - 408, and a new label, approved May 2, 2007, which limits use to one site, lettuce, and two states, Arizona and California, at the rate of 1 pound per acre. The registrant, Wilbur-Ellis, agreed to use the newly-approved label immediately for 2007 product. The old label allowed use of diazinon granular on many vegetables, as well as melons. No comments have been received on the proposed cancellation (comment period ended June 15), so the product will be cancelled. All sales of this product will cease after December 2008. This is the last remaining granular section 3 product for diazinon.

The SLN CA05000200 is registered to the California Department of Food and Agriculture, to be used for fruit fly pests subject to State Quarantine action on ornamental host and tree fruit nursery stock (all fruit must be removed from plants), ornamental nursery stock, and cannery waste drying in fields. It is labeled for use at 5 lbs. a.i. per acre. Treatments are for quarantine and eradication purposes only and limited to conduct under direct supervision by federal, state or county authorized persons. The County

Agricultural Commissioner's (or designee's) signature must be obtained prior to this use. This product is for soil drench under host plants. Commodities on the label which are not included on the EFED table are: fresh beans, bushberries, corn, filberts, grapes, olives, pecans, squash, and walnuts.

EFED Table For Diazinon in Memorandum of June 18, 2007

Use	Max Ap. rate per application (lbs a.i./A)	Maximum # of applications per year	Application method(s)
Outdoor ornamentals	1	26*	foliar
Almonds	3	1	foliar or dormant
Apples	2	2	1 dormant + 1 foliar; or 2 foliar
Apricots	2	2	1 dormant + 1 foliar
Blueberries	1	2	1 in season foliar + 1 fire ant control
Caneberries	2	1	foliar
Cherries	2	2	1 dormant + 1 foliar
Fig	0.5	1	unclear
Nectarines	2	2	1 dormant + 1 foliar
Peaches	2	2	1 dormant + 1 foliar
Pears	2	2	1 dormant + 1 foliar
Plums	2	2	1 dormant + 1 foliar
Prunes	2	2	1 dormant + 1 foliar
Strawberries	1	2	1 foliar + 1 soil (incorporation)
Beans (succulent)	4	1	soil (incorporation)
Beets, Red	4	1	soil (incorporation)
Broccoli	4	1	soil (incorporation)
Brussels Sprouts	4	1	soil (incorporation)
Cabbage	4	1	soil (incorporation)
Carrots	4	1	soil (incorporation)
Cauliflower	4	1	soil (incorporation)
Collards	4	1	soil (incorporation)
Endive	4	1	soil (incorporation)
Kale	4	1	soil (incorporation)
Lettuce	2	2**	1 soil (incorporation) + 1 foliar, aerial permitted
Melons	4	2	1 soil (incorporation) + 1 foliar
Mustard greens	4	1	soil (incorporation)
Onions	4	1	soil (incorporation)
Peas (succulent)	4	1	soil (incorporation)
Peppers (bell and chili)	4	1	soil (incorporation)
Radishes	4	1	soil (incorporation)
Rutabagas	4	1	soil (incorporation)
Spinach	4	1	soil (incorporation)
Sweet potatoes	4	1	soil (incorporation)
Tomatoes	4	1	soil (incorporation)

*The label specifies a maximum application rate per season. It is assumed that it is possible to have 26 seasons per year.
**The label specifies a maximum application rate per season. It is assumed that it is possible to have more than one season of lettuce per year.

Appendix K. Detailed analysis of final diazinon action area and overlap of action area with CRLF core areas and critical habitat

K.1. Currently registered uses of diazinon

Currently, labeled uses of diazinon include several fruit, nut, and vegetable crops as well as cattle ear tags. There are 14 active Section 3 labels of products containing diazinon. The EPA registration numbers for these labels are 2935-408, 4581-392, 5905-248, 19713-91, 19713-492, 66222-9, 66222-10, 66222-103, 11556-123, 39039-3, 39039-6, 61483-78, 61483-80, and 61483-92. In addition, there are 3 special local needs labels for California: CA-000030, CA-030014 and CA-050002. A comprehensive list of these uses included in Table K.1. These uses are the basis for determining the initial action area for areas where diazinon can be directly applied.

Table K.1 Currently registered uses of diazinon.

Category	Use
Agriculture	Beans (succulent), beets (red), blueberries, broccoli, Brussels sprouts, cabbage, caneberries, carrots, cauliflower, collards, endive, ginseng, kale, lettuce, melons, mustard, onions, outdoor ornamental, peas (succulent), peppers, potatoes, radishes, rutabagas, spinach, squash, strawberries, sweet corn, tomatoes
Orchard	Almonds, Apples, apricots, cherries, cranberries, nectarines, peaches, pears, pineapple, plums, prunes

K.2. Determination of area where diazinon is potentially directly applied (initial area of concern)

After determination of which uses will be assessed, an evaluation of the potential “footprint” of the use pattern is determined. This “footprint” represents the initial area of concern and is typically based on available land cover data. Local land cover data available for the state of California were analyzed to refine the understanding of potential diazinon use. The initial area of concern is defined as all land cover types that represent the labeled uses described above. The initial area of concern is represented by 1) agricultural landcovers, which are assumed to represent vegetable and non-orchard fruit crops as well as ornamental crops and 2) orchard and vineyard landcovers which are assumed to be representative of tree fruit and almond crops.

Base mapping layers for determining the initial area of concern were obtained from the National Land-cover Dataset (NLCD 2001) for the majority of land use types and the California GAP data (6/98) for the orchards and vineyard uses. The NLCD is a recently released national land use dataset and the GAP is from the Biogeography Lab from UCLA-Santa Barbara. These raster files were converted to vector and used in the analysis. **Table K.2** shows the land-cover sources used.

Table K.2. Land-cover data sources used for determining areas where diazinon is potentially applied.

Land-cover Data Sources			
Layer name	**Base source**	**Description**	**non-NASS**
Cultivated Crops	NLCD	82: Areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20 percent of total vegetation. This class also includes all land being actively tilled.	No
Developed, High Intensity	NLCD	24: Includes highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80 to100 percent of the total cover.	Yes
Developed, Low Intensity	NLCD	22: Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20-49 percent of total cover. These areas most commonly include single-family housing units.	Yes
Developed, Medium Intensity	NLCD	23: Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50-79 percent of the total cover. These areas most commonly include single-family housing units.	Yes
Developed, Open Space	NLCD	21: Includes areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20 percent of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.	Yes
Forest	NLCD	Union of 41,42,43: Deciduous, evergreen and mixed. Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover.	Yes
Open Water	NLCD	11: All areas of open water, generally with less than 25% cover of vegetation or soil.	Yes
Orchards and vineyards	CA GAP	A union of 11210, 11211 and 11212. This is the only CA GAP reference.	No
Pasture/Hay	NLCD	81: Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20 percent of total vegetation.	No
Wetlands	NLCD	Union of 90, 95: Woody wetlands and emergent herbaceous.	Yes

U.S. Department of Agriculture's National Agriculture Statistics Service (NASS) census dataset, 2002 was used to determine whether a crop was grown in a particular county. This census dataset provides survey information over five years on agricultural practices and is used mainly for cultivated or agriculture crops. Chemical labeled uses were matched to NASS uses; an agriculture use match would result in a mapped area for one or more counties.

In counties where a use has been identified, the use is associated with the appropriate landcover data set (Table K.2). It is assumed that this use is potentially grown in the area where the landcover has been identified. For example, almonds are grown in Kern County. According to CA GAP data, there are orchards or vineyards within Kern County. Therefore, it is assumed that almonds, which are grown in orchards, can be grown on any of the land specified by the CA GAP data as orchards or vineyards. This process is carried out for almonds in every county in California.

Uses of diazinon fall into two landcover categories: cultivated crops and orchards and vineyards. Therefore, two separate action area maps are created for the different uses: 1) agriculture, which is based on the cultivated crop layer from NLCD and 2) orchard crops, which is based on the orchard and vineyard layer from CA GAP. Therefore, two separate action areas are defined for this assessment; one to represent crops represented by agricultural areas and the other to represent areas classified as orchards. For specific uses associated with these categories, see Table K.1. The initial agricultural and orchard action areas are depicted in Figures K.1 and K.2, respectively.

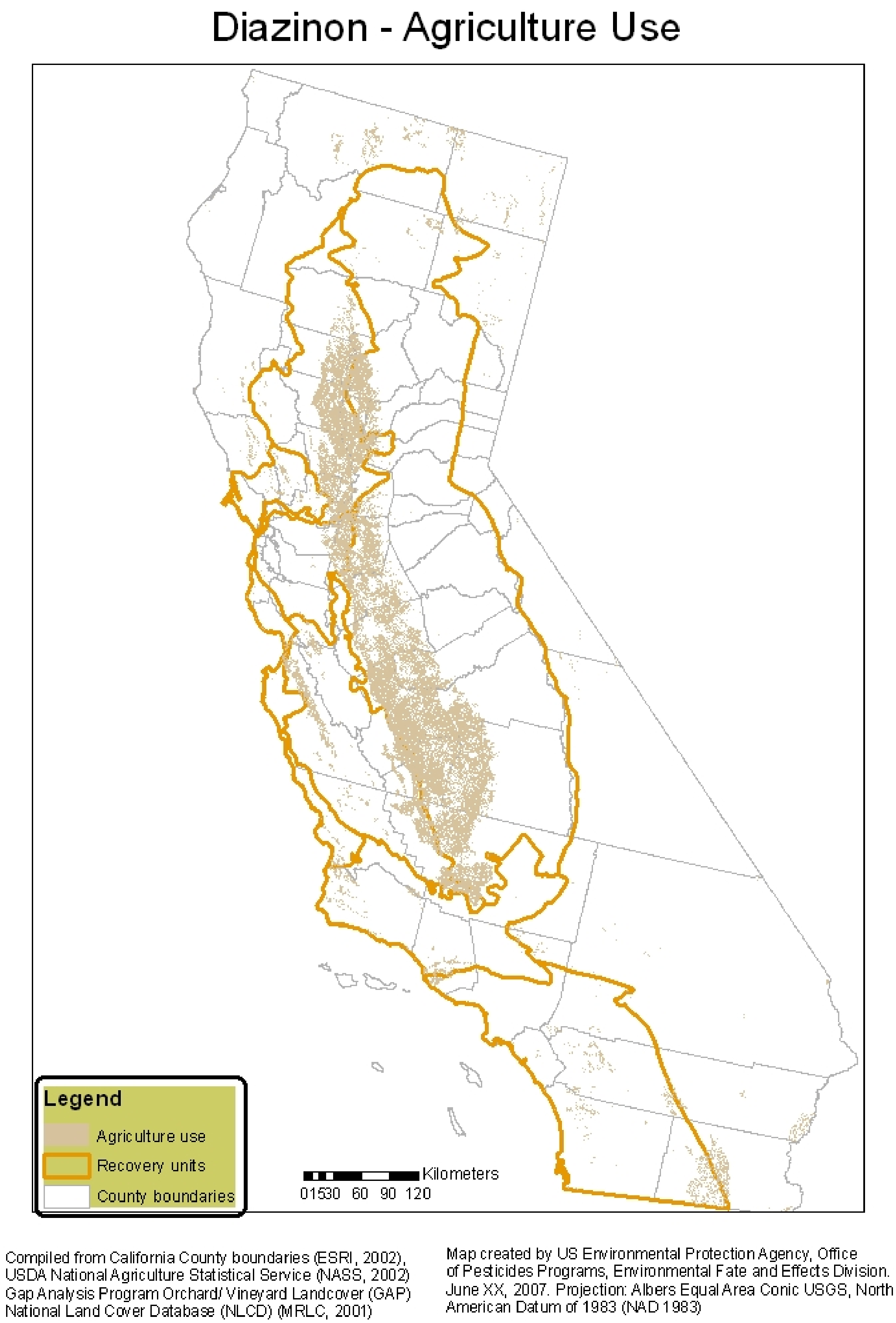


Figure K.1. Initial area of concern associated with agricultural uses.

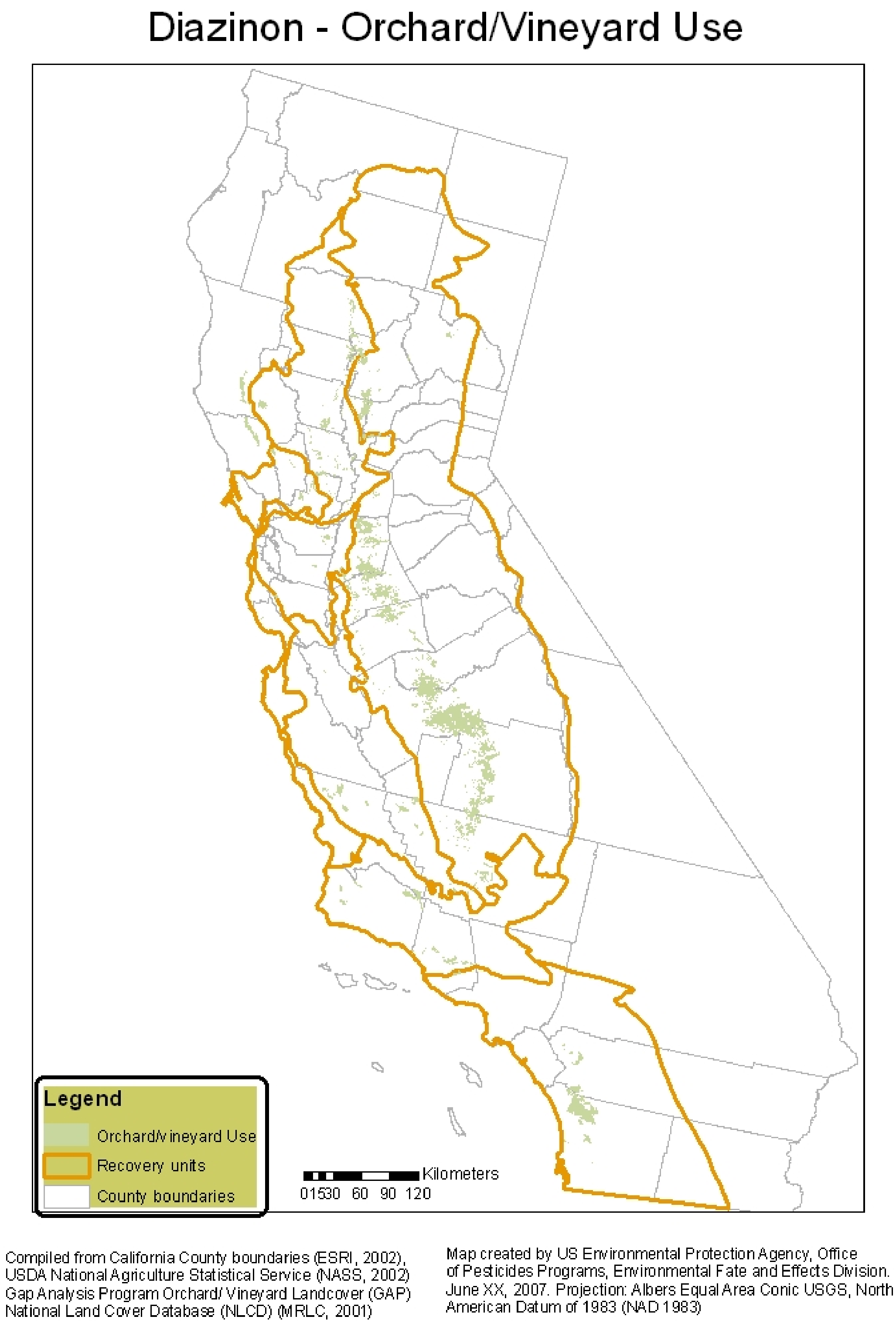


Figure K.2. Initial area of concern associated with orchard uses.

K.3. Determination of area indirectly affected by diazinon use

Since this screening level risk assessment defines taxa that are predicted to be exposed through runoff and drift to diazinon at concentrations above the Agency's Levels of Concern (LOC), there is need to expand the action area to include areas that are affected indirectly by this federal action. Two methods are employed to define the areas indirectly affected by the federal action, and thus the total action area. These are the down stream dilution assessment for determining the extent of the affected lotic aquatic habitats (flowing) and the spray drift assessment for determining the extent of the affected terrestrial habitats. In order to define the final action areas relevant to uses of diazinon on agricultural and orchard crops, it is necessary to combine areas directly affected, as well as aquatic and terrestrial habitats indirectly affected by the federal action. It is assumed that lentic aquatic habitats (e.g. ponds, pools, marshes) overlapping with the terrestrial areas are also indirectly affected by the federal action.

The downstream extent includes the area where the EEC could potentially be above levels that would exceed the most sensitive LOC. The model calculates two values, the dilution factor (DF) and the threshold Percent Cropped Area (PCA). The dilution factor (DF) is the maximum RQ/LOC, and the threshold PCA is the inverse value represented as a percent.

The dilution model uses the NHDPlus data set (http://www.horizon-systems.com/nhdplus/) as the framework for the downstream analysis. The NHDPlus includes several pieces of information that can be used to analyze downstream effects. For each stream reach in the hydrography network, the data provide a tally of the total area in each NLCD land cover class for the upstream cumulative area contributing to the given stream reach. Using the cumulative land cover data provided by the NHDPlus, an aggregated use class is created based on the classes listed in Table K.2. A cumulative PCA is calculated for each stream reach based on the aggregate use class (divided by the total upstream contribution area).

The dilution model traverses downstream from each stream segment within the initial area of concern. At each downstream node, the threshold PCA is compared to the aggregate cumulative PCA. If the cumulative PCA exceeds the threshold then the stream segment is included in the downstream extent. The model continues traversing downstream until the cumulative PCA no longer exceeds the threshold.

In order to determine the extent of the action area in lotic (flowing) aquatic habitats, the agricultural and orchard uses resulting in the greatest ratios of the RQ to the LOC for any endpoint for aquatic organisms is used to determine the distance downstream for concentrations to be diluted below levels that would be of concern (i.e. result in RQs above the LOC). For this assessment, the greatest ratio for an agricultural use is 5665, for indirect effects to the CRLF through acute effects to aquatic invertebrates exposed to diazinon in runoff from applications to lettuce. For an orchard crop, the greatest ratio is 1489, for indirect effects to the CRLF through acute effects to aquatic invertebrates exposed to diazinon in runoff from a single dormant season application of diazinon to almonds. The areas indirectly affected by the federal action due to runoff of diazinon to aquatic habitats are depicted in **Figures K.3 and K.4**. The total number of kilometers of stream miles within the action area that are at levels of concern are defined in **Table K.3**.

Table K.3. Quantitative results of spatial analysis of lotic aquatic action area relevant to diazinon.

Measure	Distance (km)	
	Agricultural Areas	Orchard Areas
Total Streams in CA	332,962	332,962
Streams within initial area of concern	57,087	11,945
Downstream distance added	20,027	3,522
Streams in aquatic action area	77,114	15,467

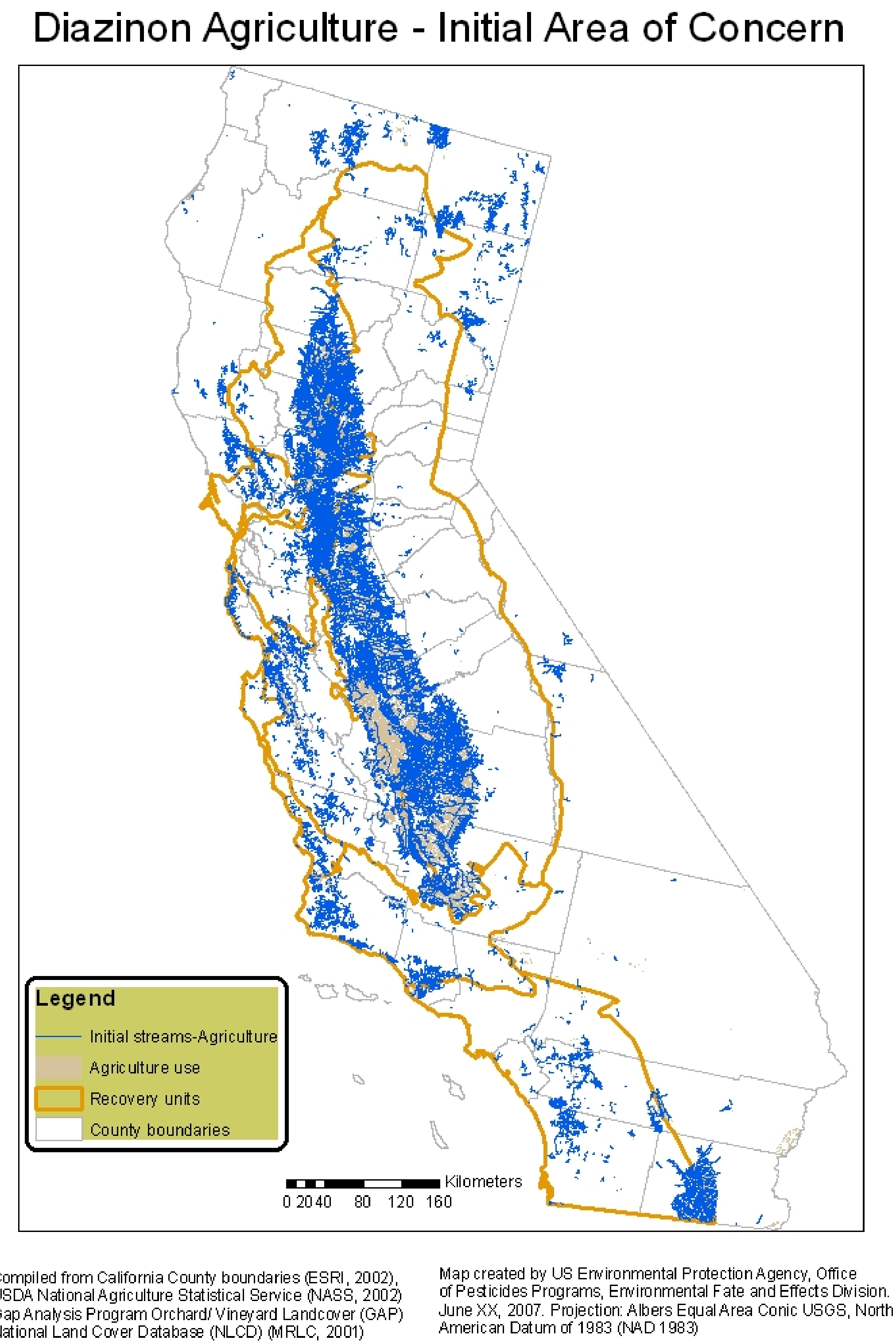


Figure K.3. Miles of streams at levels of concern resulting from diazinon applications of diazinon to agricultural uses.

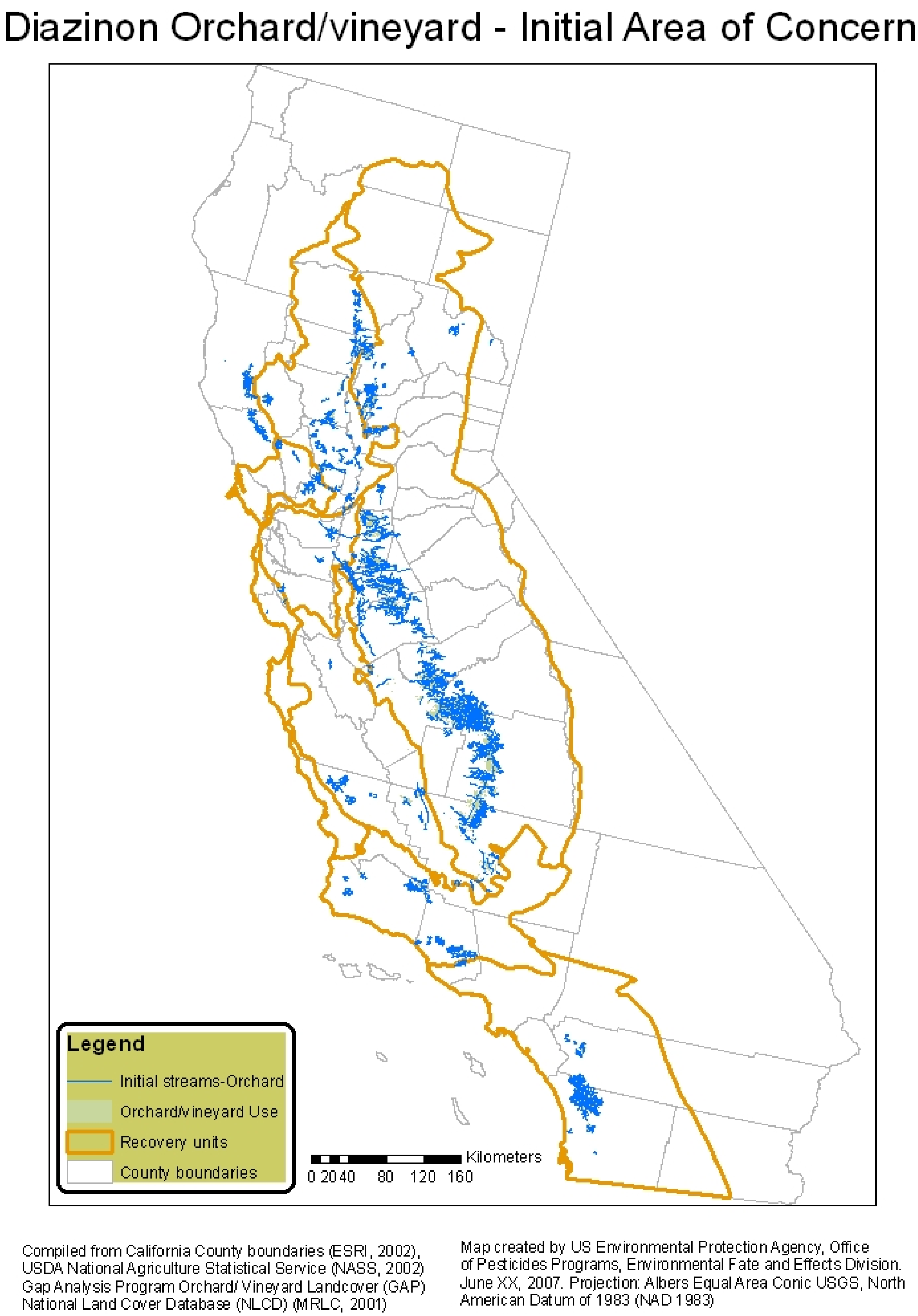


Figure K.4. Miles of streams at levels of concern resulting from diazinon applications of diazinon to orchard uses.

When considering the terrestrial habitats of the CRLF, spray drift from use sites onto non-target areas could potentially result in exposures of the CRLF, its prey and its habitat to diazinon. Therefore, it is necessary to estimate the distance from the application site where spray drift exposures do not result in LOC exceedances for organisms within the terrestrial habitat.

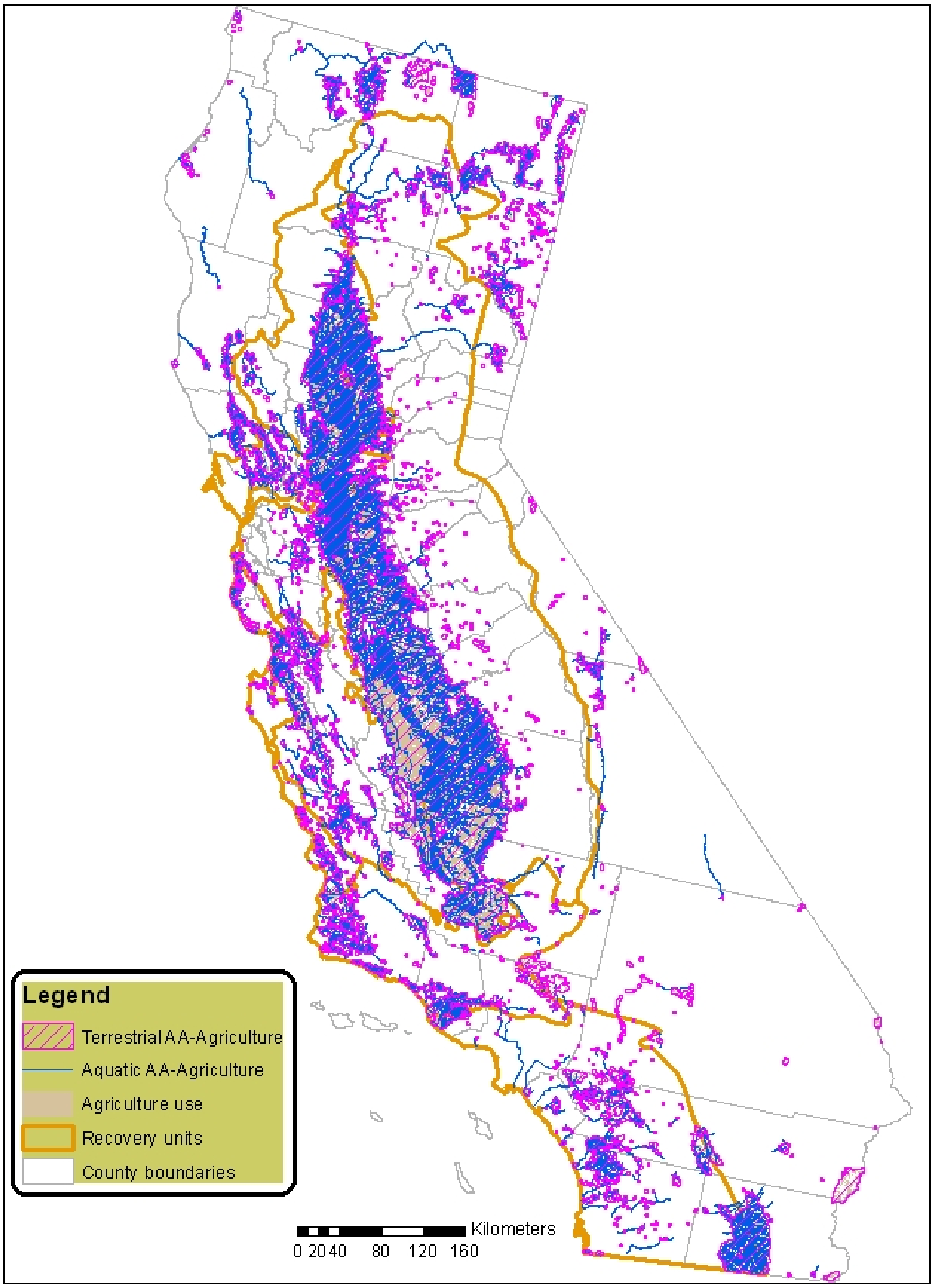
To account for this, first, the diazinon application rate which does not result in an LOC exceedance is calculated for each terrestrial taxa of concern. The lowest application rate for terrestrial organisms (0.0005 lbs a.i./A), which is relevant to direct effects to CRLF through acute, dose-based exposures, is selected for determining the concentration of diazinon in spray drift that will not result in an LOC exceedance. AgDRIFT and AGDISP are then used to estimate the distance from the edge of the field of an application site where the concentration will reach 0.0005 lbs a.i./A, indicating no LOC exceedances for terrestrial organisms. The input parameters and detailed results are described in section 3.2.3. For agricultural crops, the maximum distance from the edge of field is 11,617 feet (2.2 miles), which was estimated based on aerial application to lettuce at the maximum single application rate (2 lbs a.i./A). For orchard crops, the maximum distance from the edge of field required to result in no LOC exceedances is 933 feet, which was estimated based on airblast applications to almond crops at the maximum single application rate (3 lbs a.i./A).

To understand the area indirectly affected by the federal action due to spray drift from application areas, the landcovers where agricultural and orchard crops are grown are considered potential application areas. These areas are "buffered" using ArcGIS 9.1. In this process, the original landcover is modified by expanding the border of each polygon representing a field out to a designated distance, which in this case, is the distance estimated where diazinon in spray drift does not exceed any LOCs. This effectively expands the action area relevant to terrestrial habitats so that it includes the area directly affected by the federal action, and the area indirectly affected by the federal action. For diazinon use in agricultural areas, the agricultural use area is buffered using a distance of 11,617 feet (2.2 miles). For diazinon use in orchards, the orchard use area is buffered using a distance of 933 feet.

K.4. Determination of final action area for diazinon use

In order to define the final action areas relevant to uses of diazinon on agricultural and orchard crops, it is necessary to combine areas directly affected, as well as aquatic and terrestrial habitats indirectly affected by the federal action. This is done separately for agricultural and orchard uses using ArcGIS 9.1. Landcovers representing areas directly affected by diazinon applications are overlapped with indirectly affected aquatic habitats (determined by down stream dilution modeling) and with indirectly affected terrestrial habitats (determined by spray drift modeling). It is assumed that lentic aquatic habitats (e.g. ponds, pools, marshes) overlapping with the terrestrial areas are also indirectly affected by the federal action. The result is a final action area for diazinon uses in agricultural areas (**Figure K.5**) and a final action area for diazinon uses in orchards (**Figure K.6**).

Diazinon Agriculture - Action Area



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.5. Final action area for agricultural uses of diazinon in California.

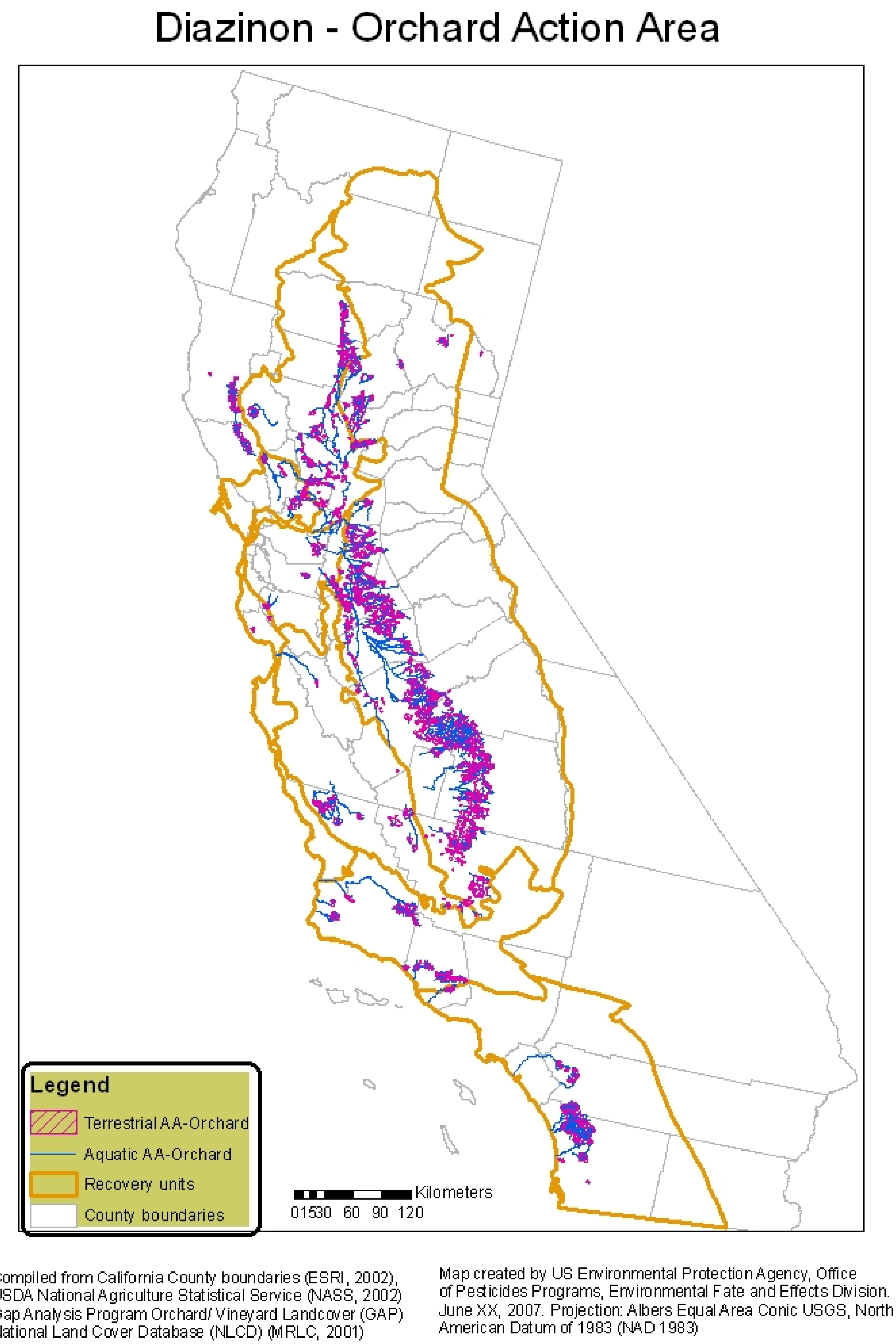
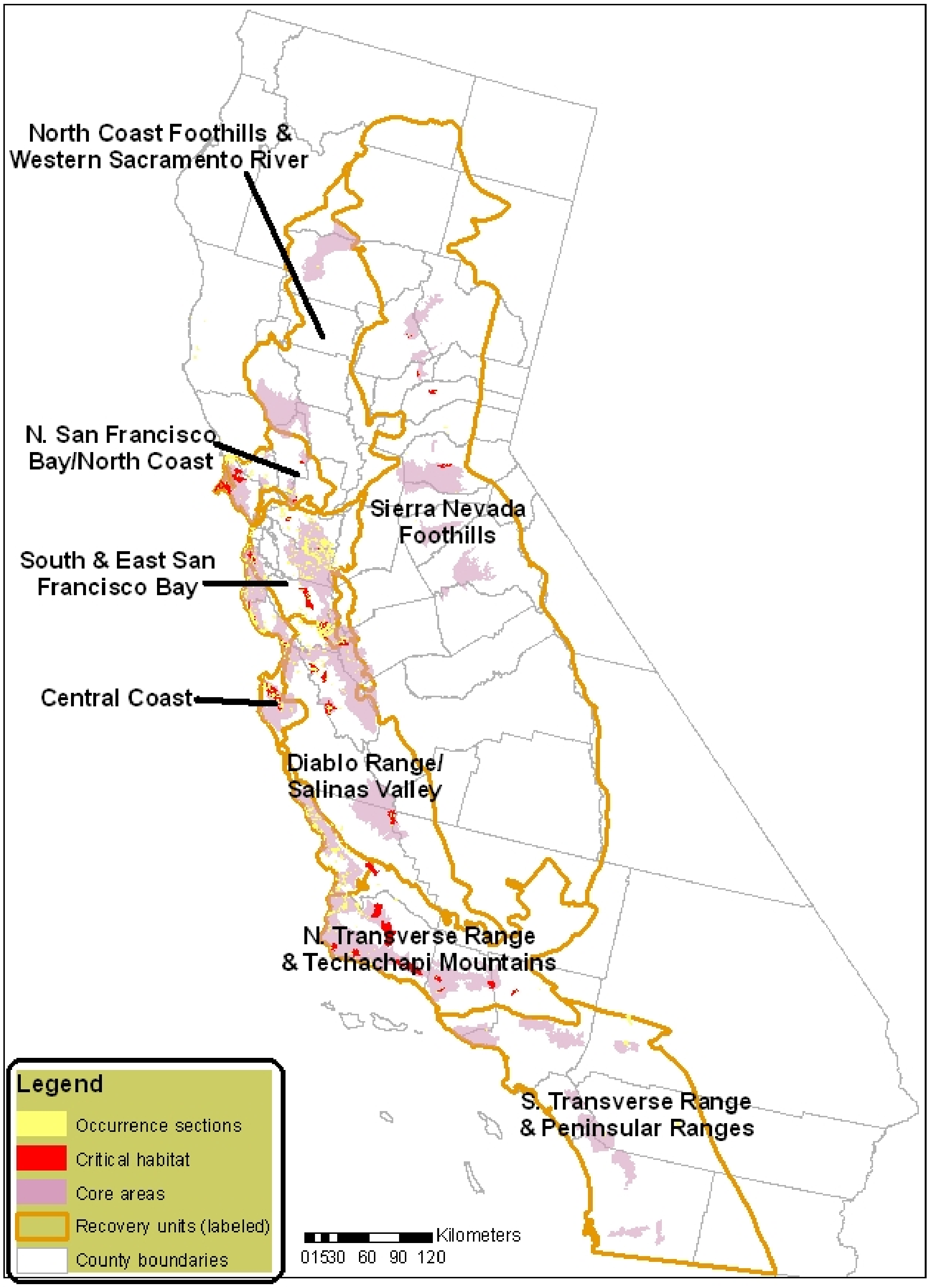


Figure K.6. Final action area for orchard uses of diazinon in California.

K.5. Determination of overlap between diazinon action area and CRLF habitat

There are three types of CRLF habitat areas considered in this assessment: Critical Habitat (CH); Core Areas; and California Natural Diversity Database (CNDDB) occurrence sections (EPA Region 9) (**Figure K.7**). Critical habitat areas were obtained from the U.S. Fish and Wildlife Service's (USFWS) final designation of critical habitat for the CRLF (USFWS 2006). Core areas were obtained from USFWS's Recovery Plan for the CRLF (USFWS 2002). The occurrence sections represent an EPA-derived subset of occurrences noted in the CNDDB. They are generalized by the Meridian Range and Township Section (MTRS) one square mile units so that individual habitat areas are obfuscated. As such, only occurrence section counts are provided and not the area potentially affected.

CRLF Habitat Areas



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June 15, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.7. Critical habitat, core areas and occurrences of CRLF.

In order to confirm that uses of diazinon have the potential to affect CRLF through direct applications to target areas and runoff and spray drift to non-target areas, it is necessary to determine whether or not the final action areas for agricultural and orchard uses of diazinon overlap with CRLF habitats. Spatial analysis using ArcGIS 9.1 indicates that lotic aquatic habitats within the CRLF core areas and critical habitats potentially contain concentrations of diazinon sufficient to exceed LOCs. In addition, terrestrial habitats (and potentially lentic aquatic habitats) of the final action areas for agricultural and orchard uses of diazinon overlap with the core areas, critical habitat and available occurrence data for CRLF (**Tables K.4 and K.5**). Thus, uses of diazinon on agricultural and orchard crops could result in exposures of diazinon to CRLF in aquatic and terrestrial habitats.

Table K.4. Overlap between CRLF habitat (core areas and critical habitat) and agricultural action area by recovery unit (RU#).

Measure	RU1	RU2	RU3	RU4	RU5	RU6	RU7	RU8	Total
CRLF habitat (km^2)*	3654	2742	1323	3279	3650	5306	4917	3326	28,197
Overlapping area of CRLF habitat and terrestrial/lentic aquatic action area (km^2)	201	158	111	535	1047	1056	1453	456	5017
% CRLF habitat overlapping with terrestrial/lentic aquatic Action Area	6%	6%	8%	16%	29%	20%	30%	14%	18%
# Occurrences overlapping with terrestrial/lentic aquatic action area	0	0	13	112	186	50	67	0	418

*Area occupied by core areas and/or critical habitat.

Table K.5. Overlap between CRLF habitat (core areas and critical habitat) and orchard action area by recovery unit (RU#).

Measure	RU1	RU2	RU3	RU4	RU5	RU6	RU7	RU8	Total
CRLF habitat (km^2)*	3654	2742	1323	3279	3650	5306	4917	3316	28,197
Overlapping area of CRLF habitat and terrestrial/lentic aquatic action area (km^2)	1.7	39	0	24	9	27	120	313	533.7
% CRLF habitat overlapping with terrestrial/lentic aquatic Action Area	0%	1%	0%	1%	0%	1%	2%	9%	2%
# Occurrences overlapping with terrestrial/lentic aquatic action area	0	0	0	11	1	1	8	0	0

*Area occupied by core areas and/or critical habitat.

Maps the overlap of CRLF core areas, critical habitat and occurrences and the total California action areas for agricultural and orchard uses of diazinon are depicted in Figures K.8, K.9, K.16 and K.17. More detailed maps of the intersection of the diazinon action area for agricultural uses are depicted in Figures K.10-K.15. More detailed maps of the intersection of the diazinon action area for orchard uses are depicted in Figures K.18-K.23.

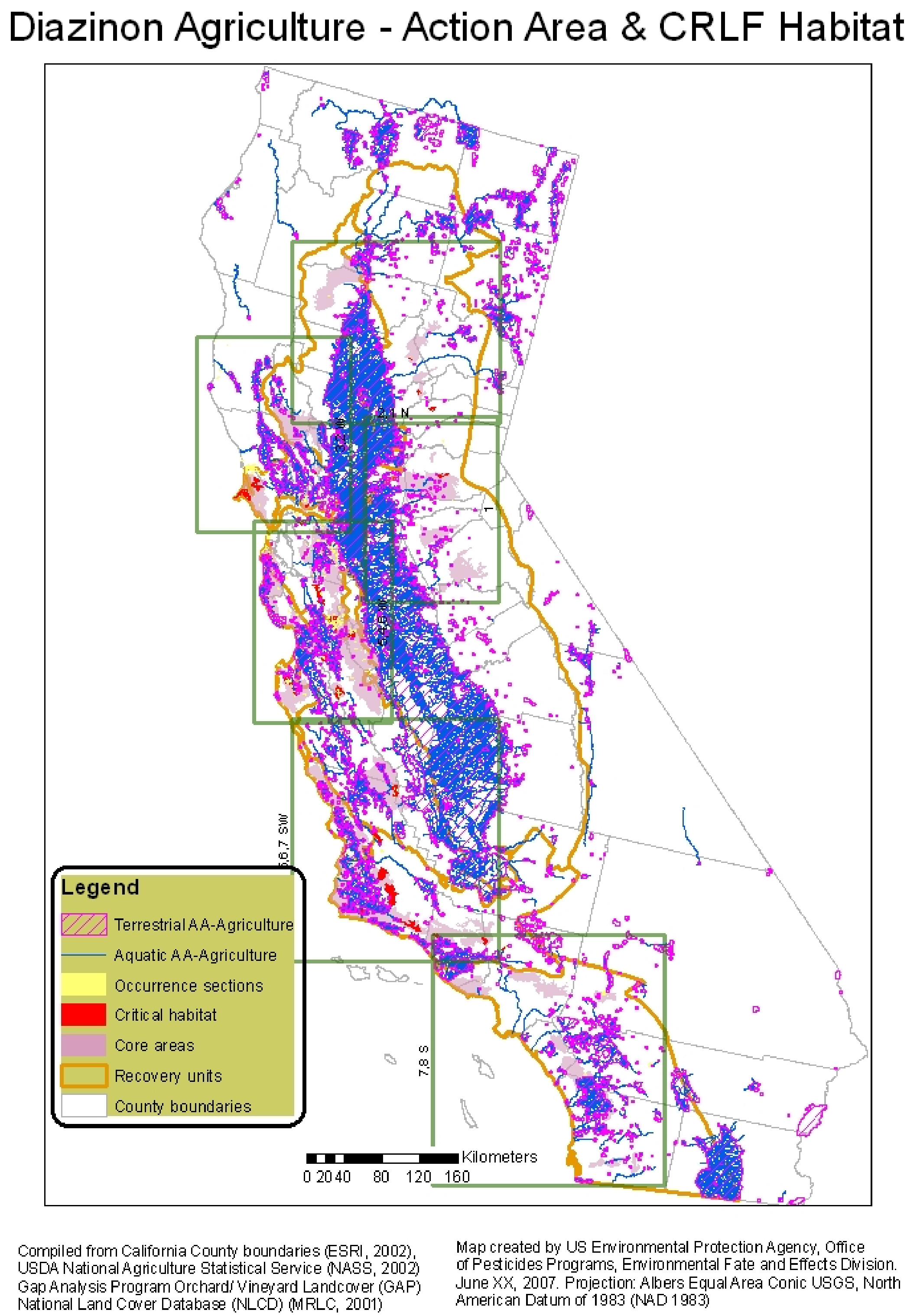


Figure K.8. Overlap of action area associated with agricultural uses of diazinon and CRLF habitat.

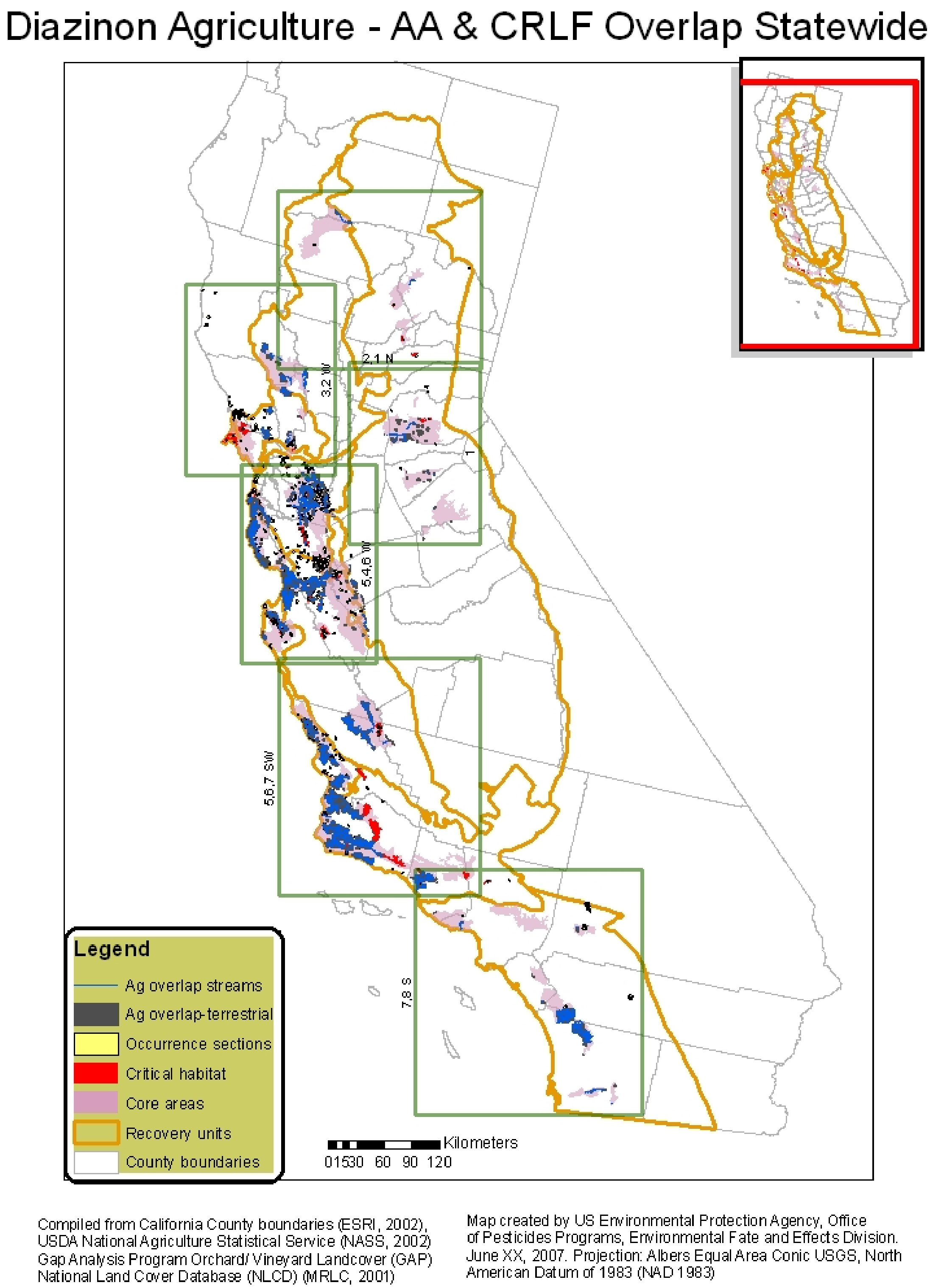


Figure K.9. Overlap of action area associated with agricultural uses of diazinon and CRLF habitat. Only areas where action area overlaps with CRLF areas are depicted. Sections depicted by green boxes are magnified in Figures K.10-K.15.

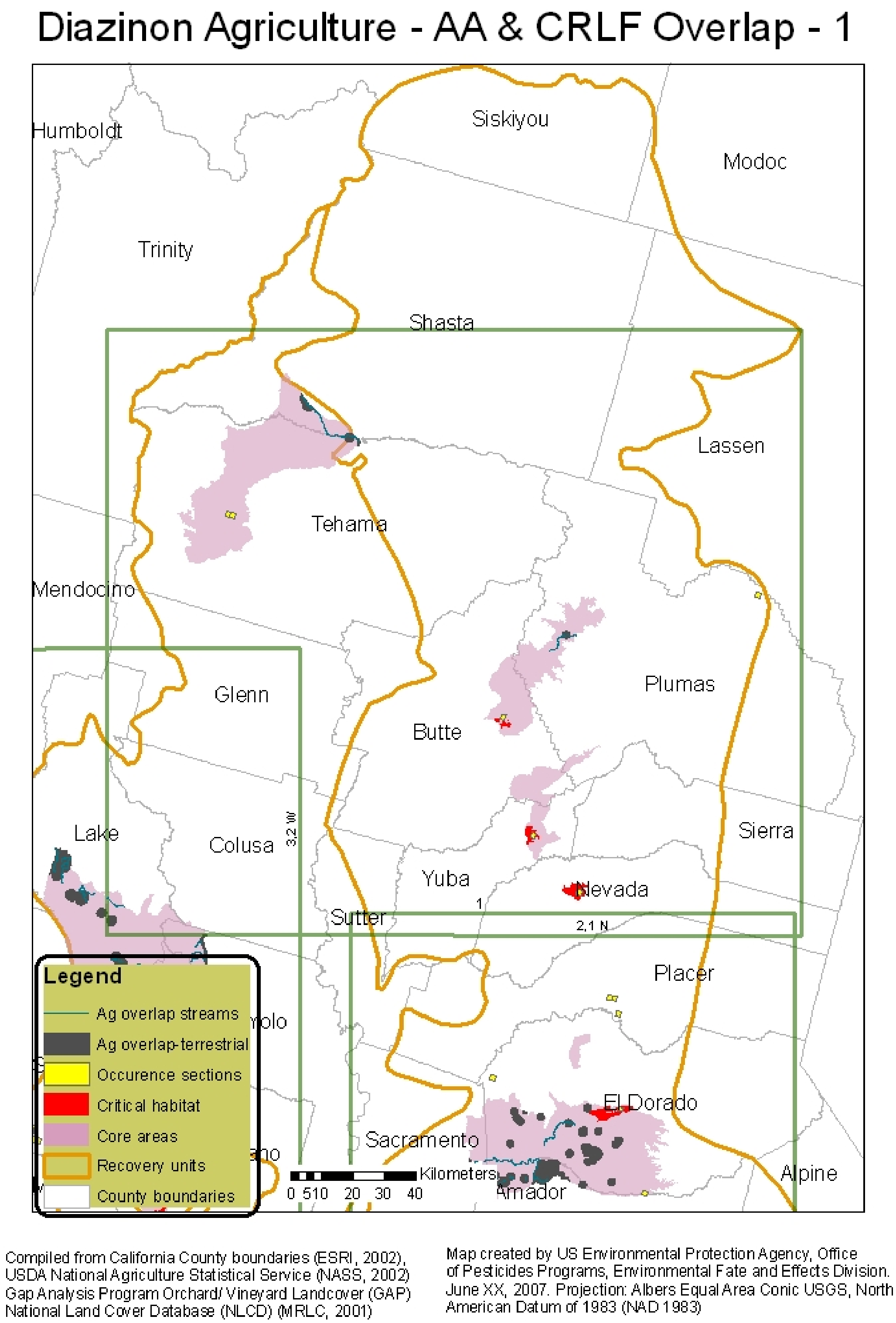
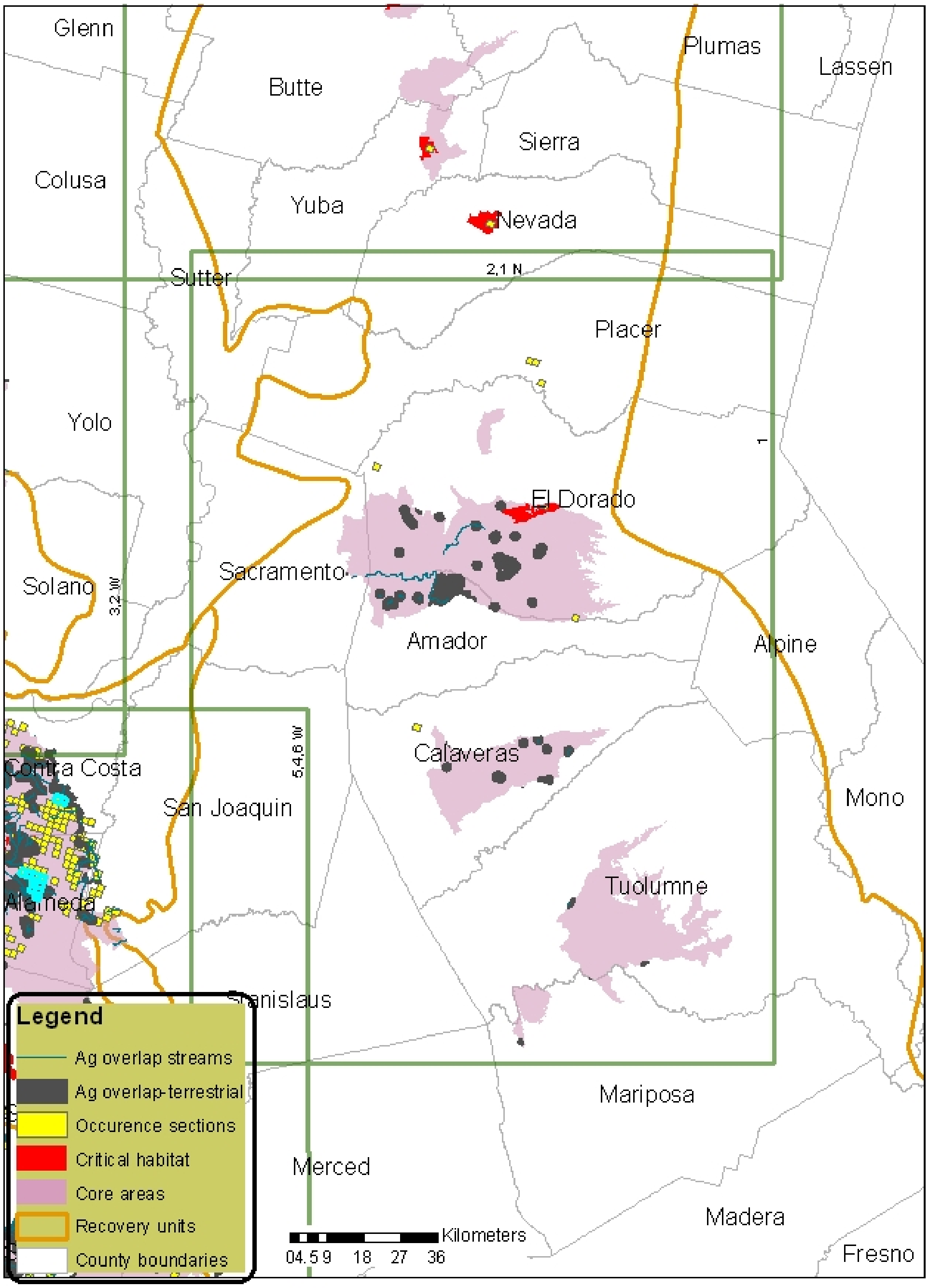


Figure K.10. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

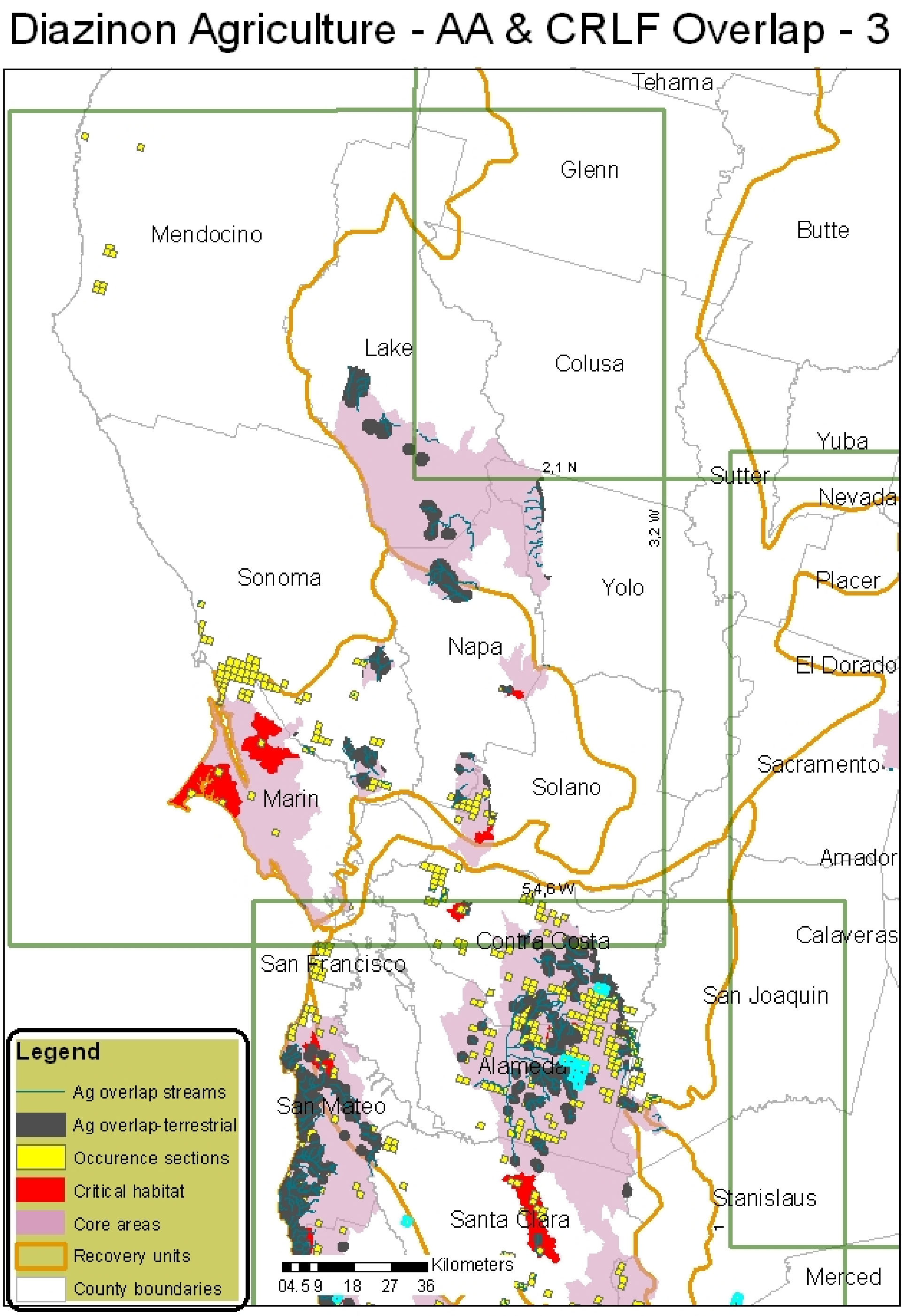
Diazinon Agriculture - AA & CRLF Overlap - 2



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.11. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

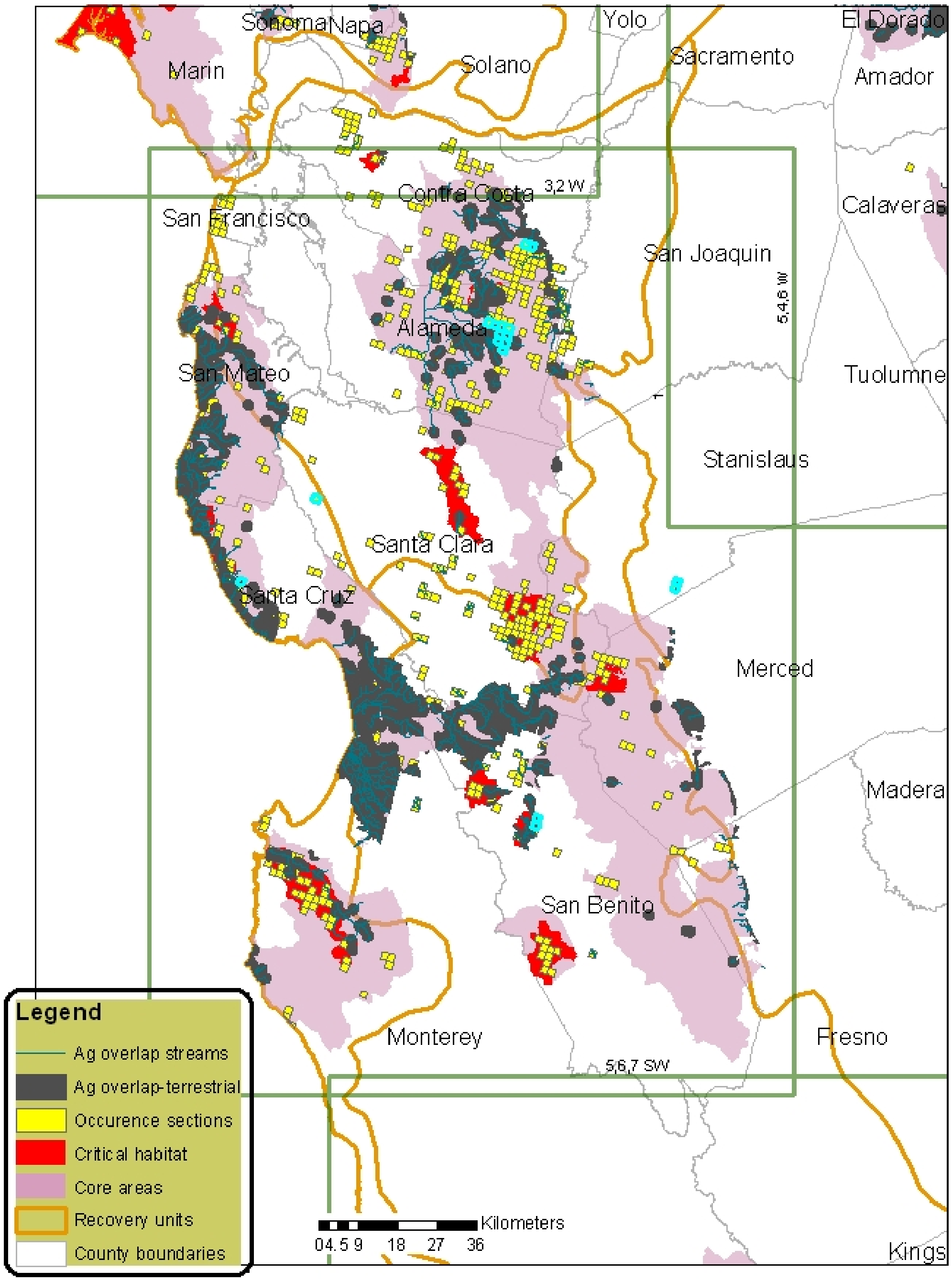


Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.12. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

Diazinon Agriculture - AA & CRLF Overlap - 4

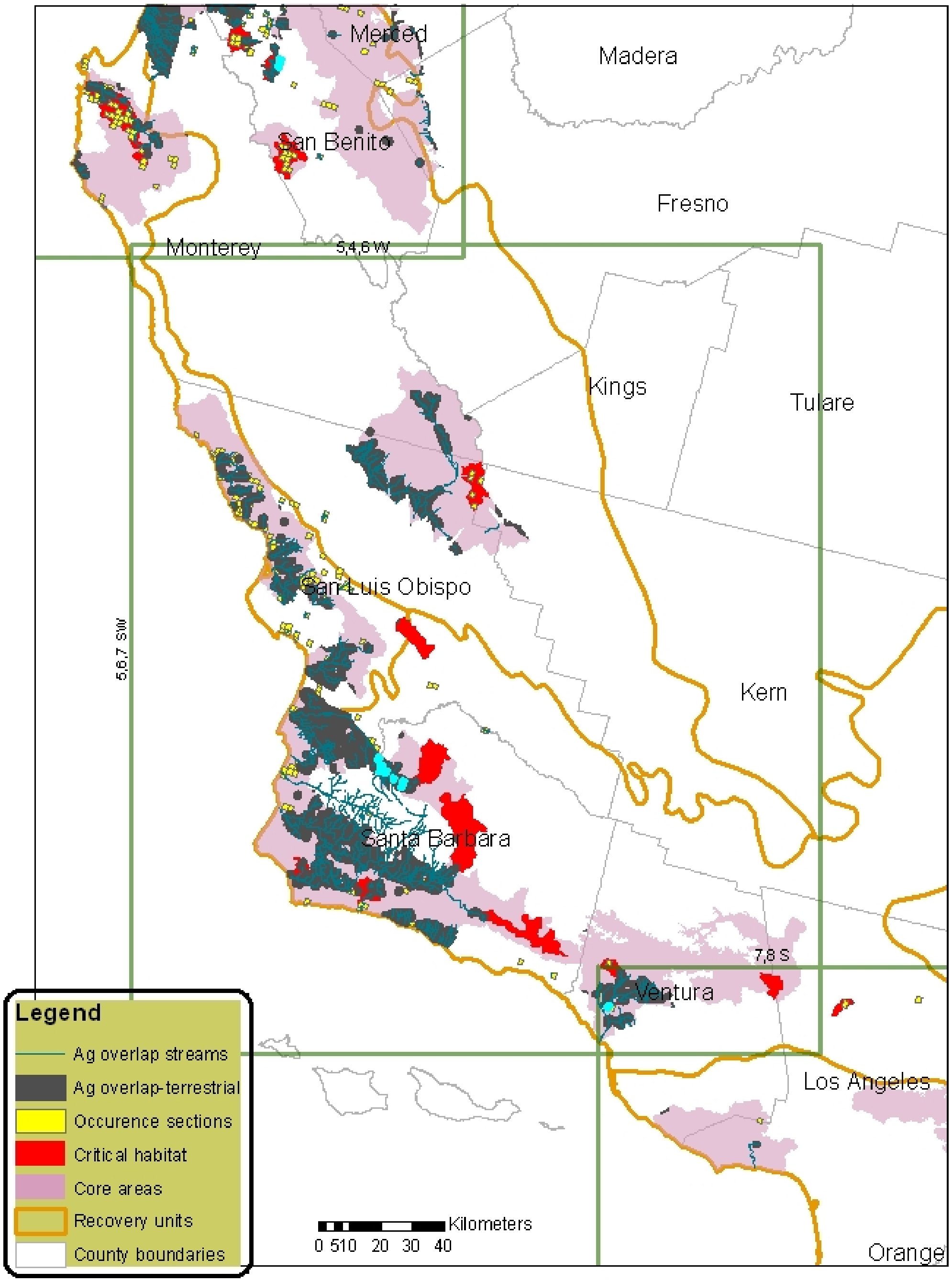


Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.13. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

Diazinon Agriculture - AA & CRLF Overlap - 5



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.14. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

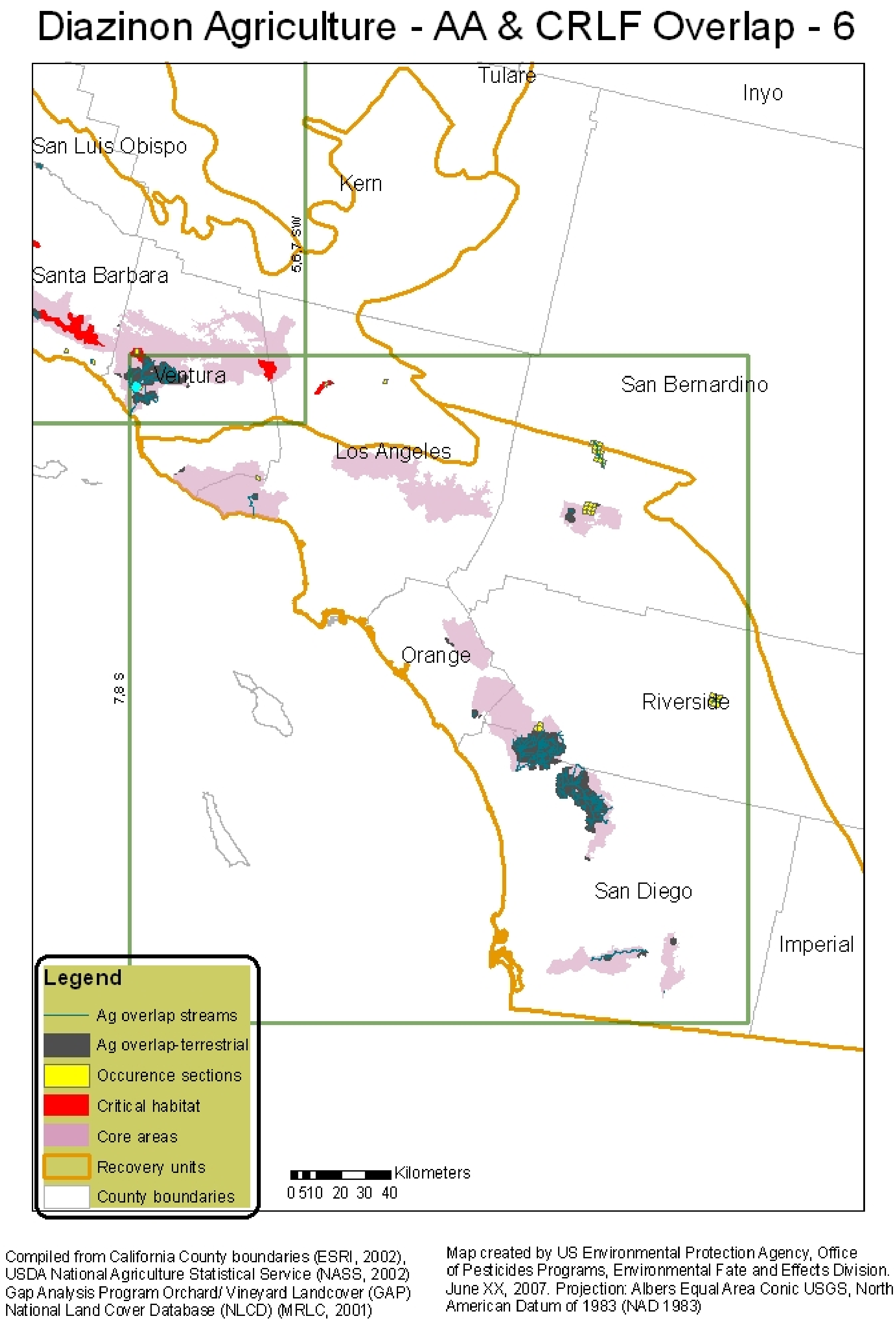


Figure K.15. Detailed map of overlap between action area for agricultural uses of diazinon and CRLF core areas and critical habitat.

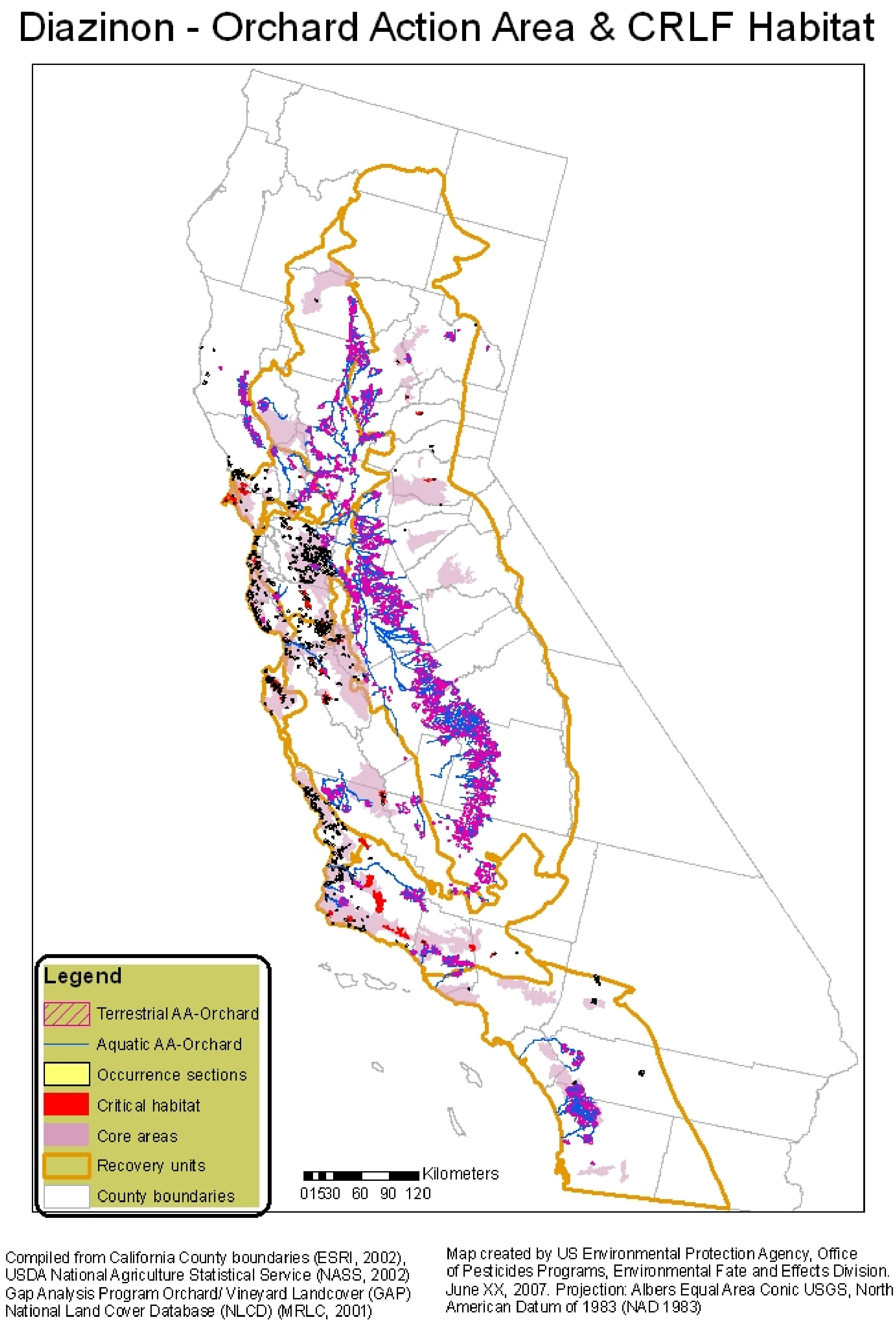


Figure K.16. Overlap of action area associated with orchard uses of diazinon and CRLF habitat.

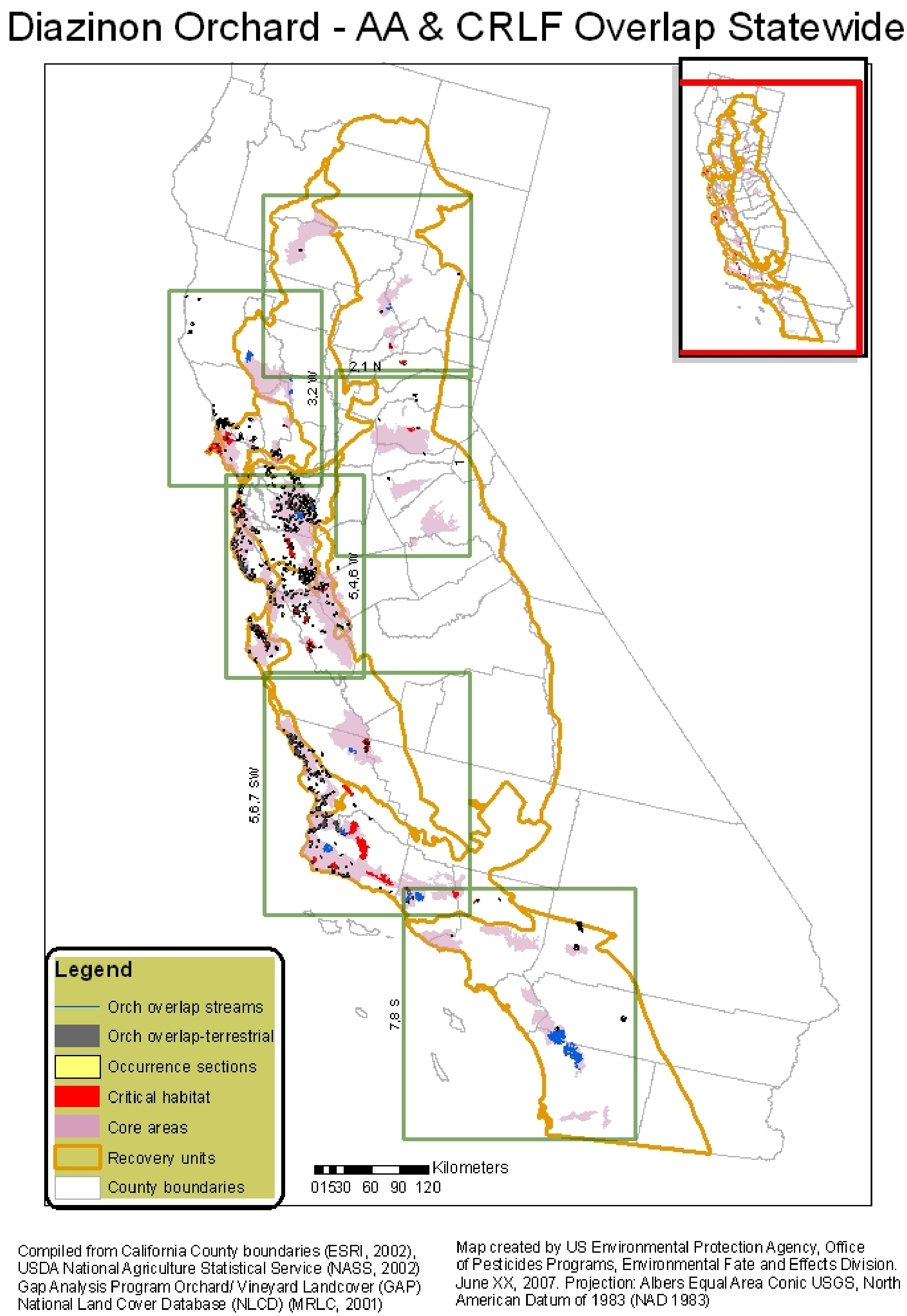


Figure K.17. Overlap of action area associated with orchard uses of diazinon and CRLF habitat. Only areas where action area overlaps with CRLF areas are depicted. Sections depicted by green boxes are magnified in Figures K.18-K.23.

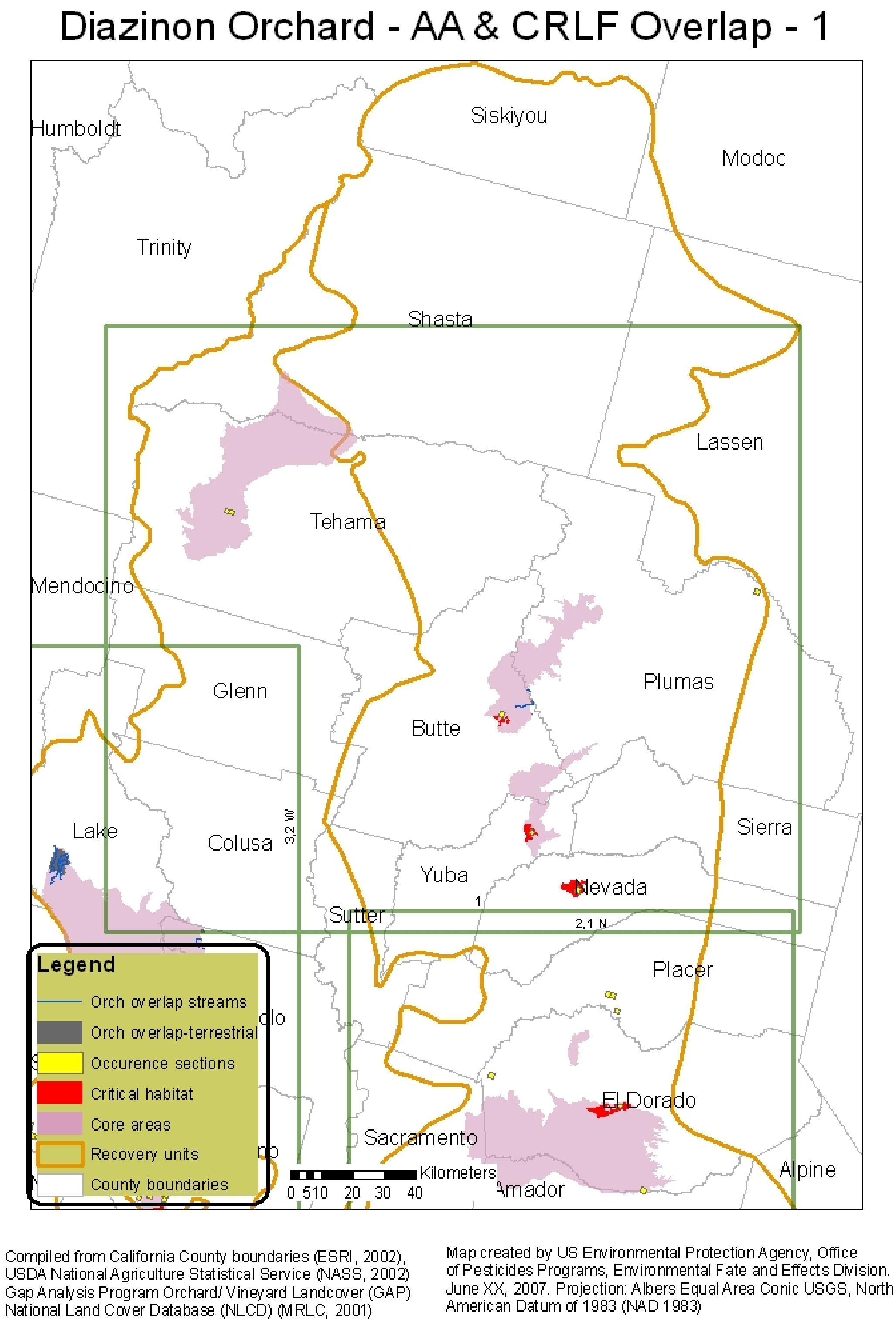
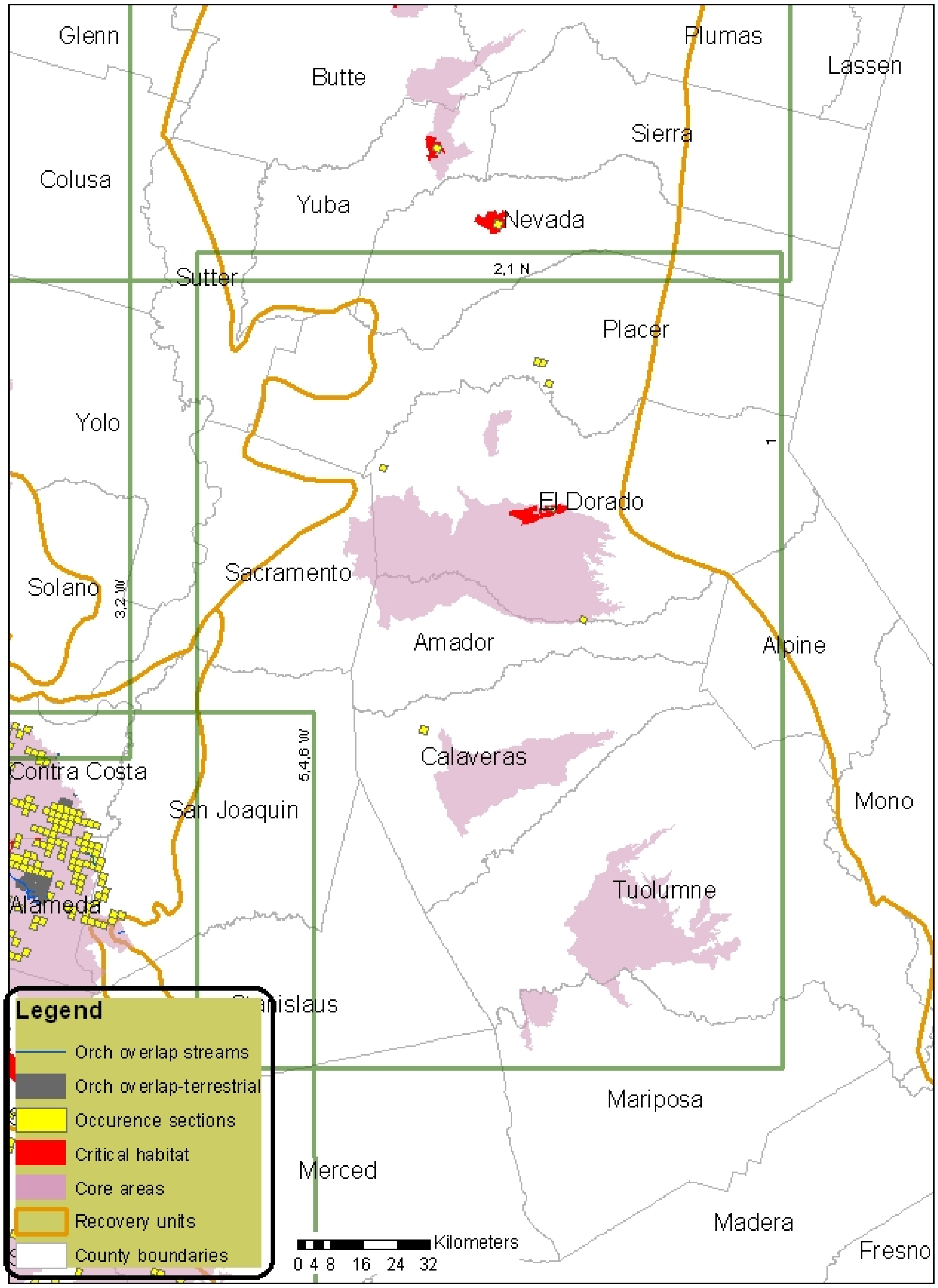


Figure K.18. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

Diazinon Orchard - AA & CRLF Overlap - 2



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure K.19. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

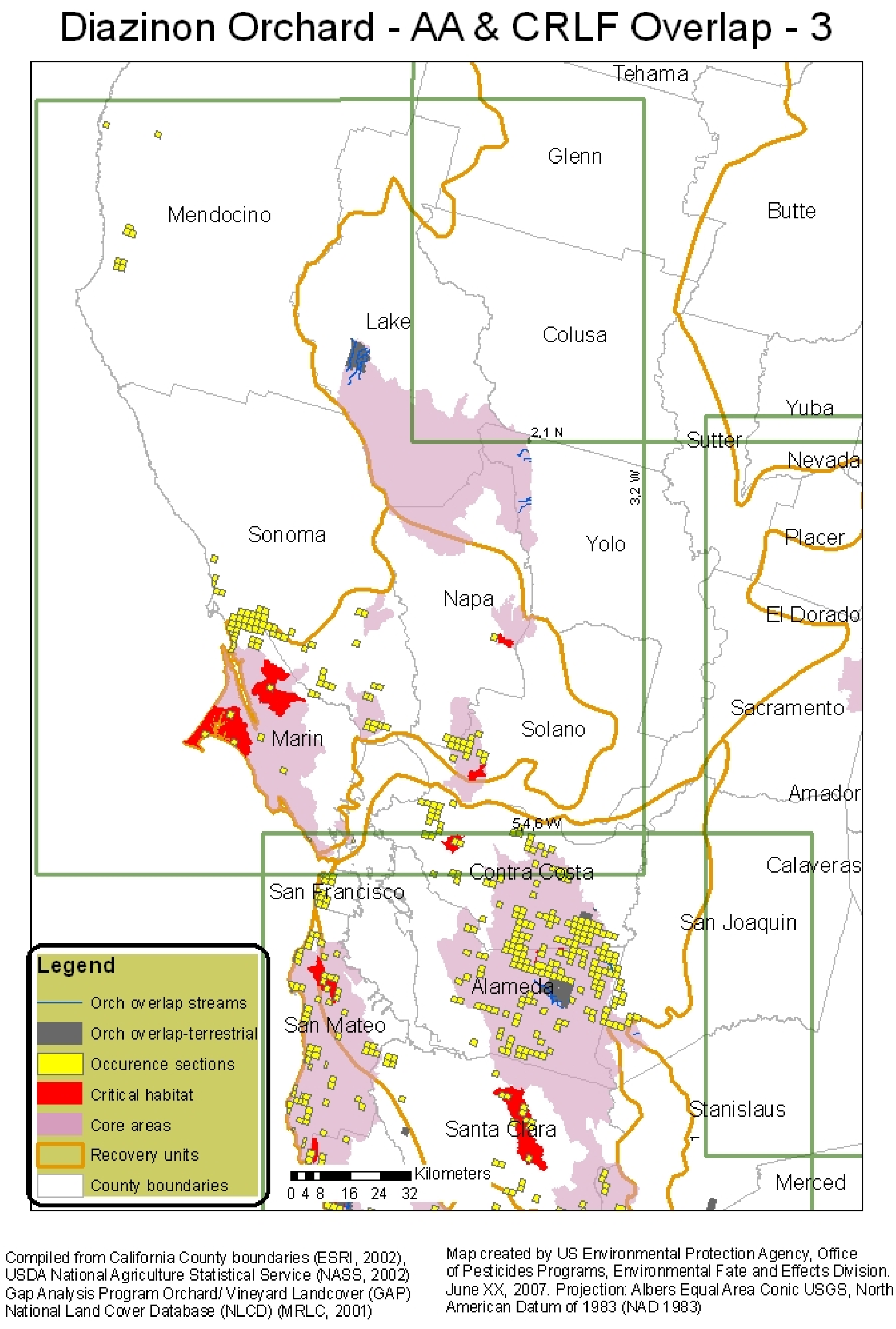


Figure K.20. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

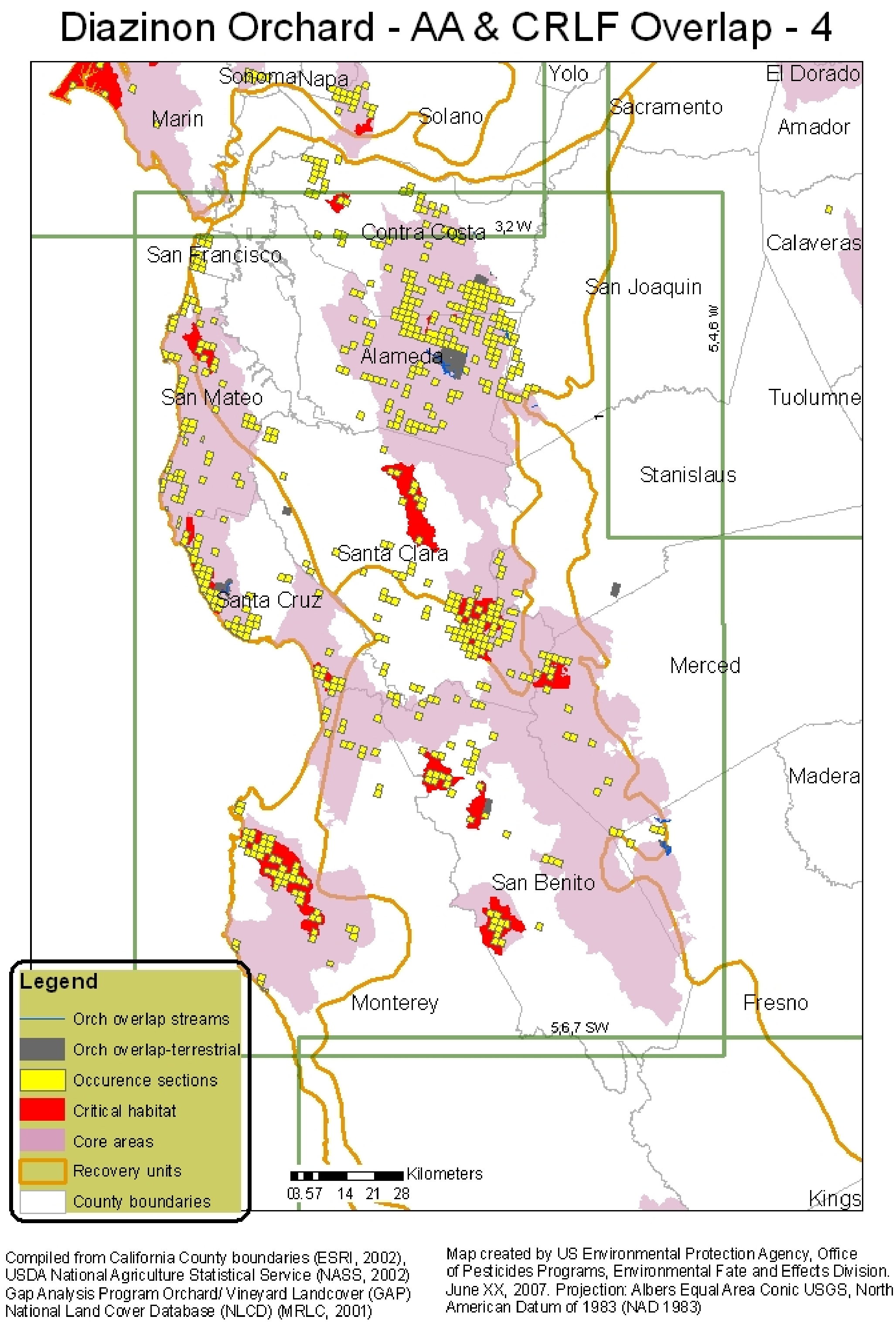


Figure K.21. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

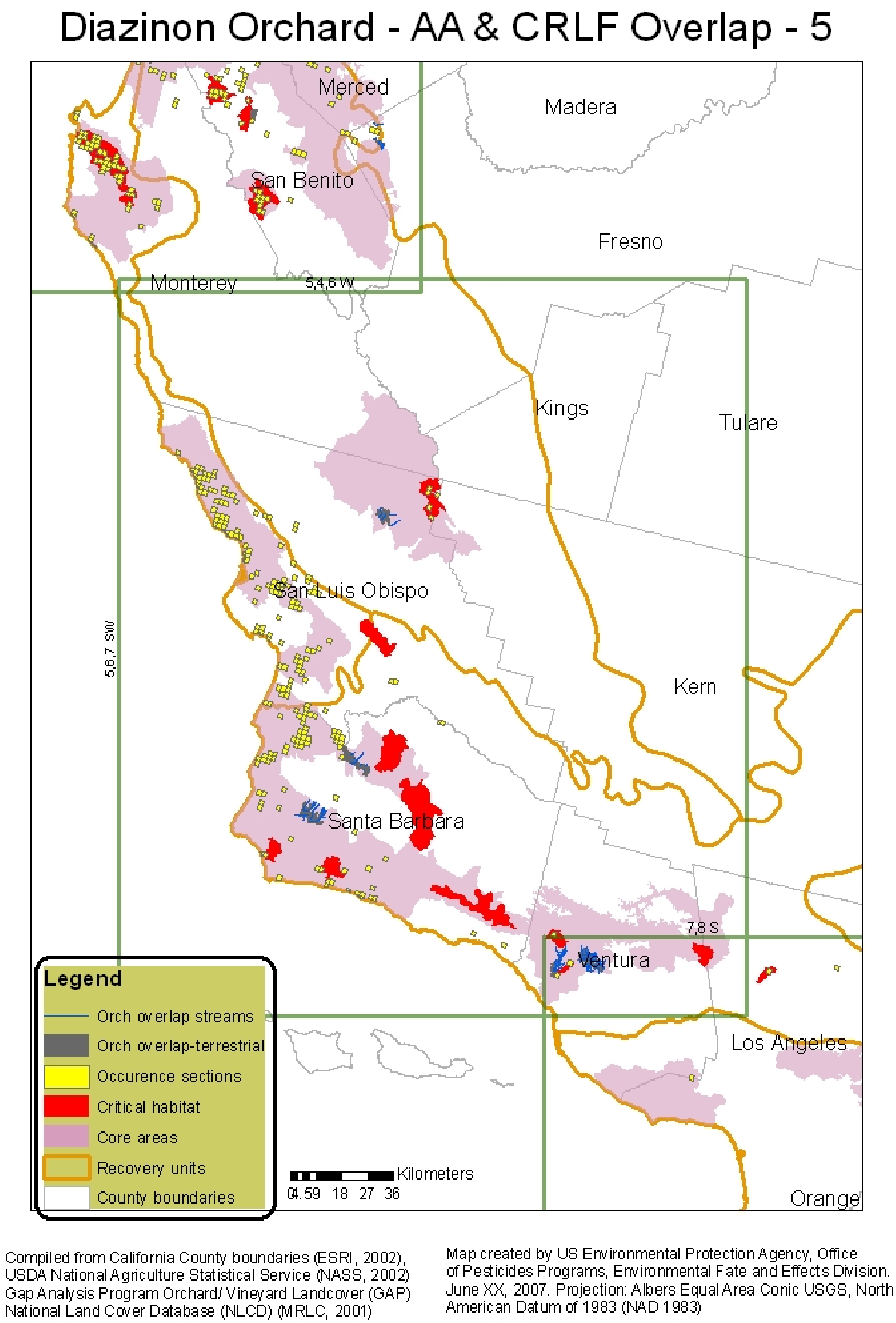


Figure K.22. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

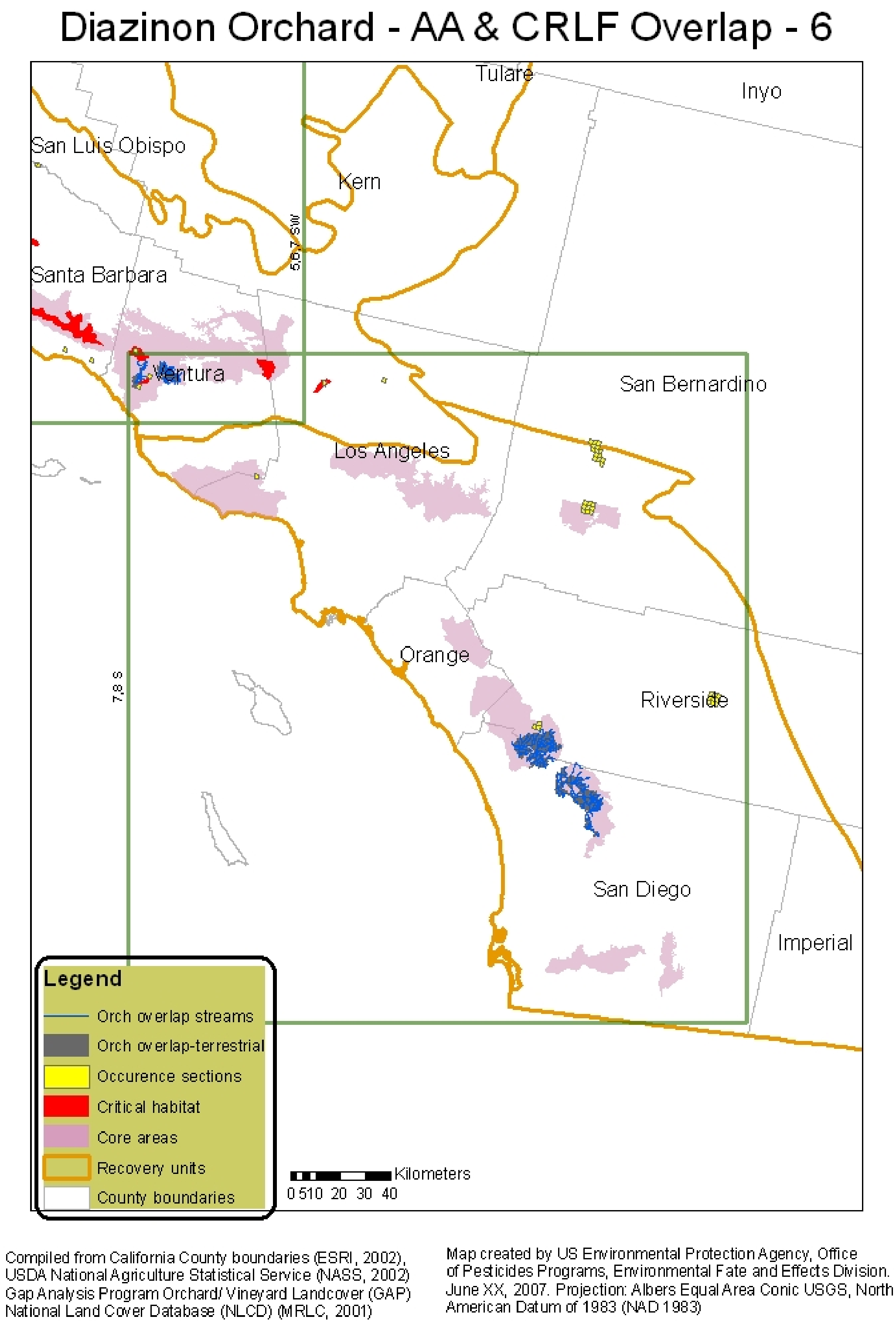


Figure K.23. Detailed map of overlap between action area for orchard uses of diazinon and CRLF core areas and critical habitat.

K.6. Limitations and Constraints of Tabular and Geospatial Sources

The geographic data sets used in this analysis are limited with respect to their accuracy and timeliness. The NASS Census of Agriculture (NASS 2002) contains adjusted survey data collected prior to 2002. Small use sites, and minor uses (e.g., specialty crops) tend to be underrepresented in this dataset. The National Land Cover Dataset (NLCD 2001) represents the best comprehensive collection of national land use and land cover information for the United States representing a range of years from 1994 – 1998. Because the NLCD does not explicitly include a class to represent orchard and vineyard landcover, California Gap Analysis Project data (CaGAP 1998) were overlaid with the NCLD and used to identify these areas.

Hydrographic data are from the NHDPlus dataset (http://www.horizon-systems.com/nhdplus/). NHDPlus contains the most current and accurate nationwide representation of hydrologic data. In some isolated instances, there are, however, errors in the data including missing or disconnected stream segments and incorrect assignment of flow direction. Spatial data describing the recovery zones and core areas are from the US Fish and Wildlife Service. The data depicting survey sections in which the species has been found in past surveys is from the California Natural Diversity Database (http://www.dfg.ca.gov/bdb/html/cnddb.html).

The relatively coarse spatial scale of these datasets precludes use of the data for highly localized studies, therefore, tabular information presented here is limited to the scale of individual Recovery Units. Additionally, some labeled uses are not possible to map precisely due to the lack of appropriate spatial data in NLCD on the location of these areas. To account for these uncertainties, the spatial analysis presented here is conservative, and may overestimate the areal extent of actual pesticide use in California.

APPENDIX L

PRODUCT FORMULATIONS CONTAINING MULTIPLE ACTIVE INGREDIENTS

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively[2] [3].

There are no product LD50 values, with associated 95% Confidence Intervals (CIs) available for diazinon.

As discussed in USEPA (2000) a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation an LD50 with associated 95% CI is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the formulated products for diazinon do not have LD50 data available it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects. However, because the active ingredients are not expected to have similar mechanisms of action, metabolites, or toxicokinetic behavior, it is reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment based on the toxicity of diazinon is the only reasonable approach that employs the available data to address the potential acute risks of the formulated products.

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[2] Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, Environmental Protection Agency (January 2004) (Overview Document).

[3] Memorandum to Office of Prevention, Pesticides and Toxic Substance, US EPA conveying an evaluation by the U.S. Fish and Wildlife Service and National Marine Fisheries Service of an approach to assessing the ecological risks of pesticide products (January 2004).

Appendix L. Pesticide Products Formulated with Diazinon and Other Pesticide Active Ingredients

DIAZINON PRODUCTS [4]

PRODUCT/TRADE NAME	EPA Reg.No.	% Diazinon	PRODUCT		ADJUSTED FOR ACTIVE INGREDIENT	
			LD 50 (mg/kg)	CI (mg/kg)	LD50 (mg/kg)	CI (mg/kg)
Co-ral plus insecticide cattle ear tag	11556-123	20	No Data	No Data	No Data	No Data
Farnam turbo	270-260	18	No Data	No Data	No Data	No Data
KMG-Bernuth	61483-92	40	No Data	No Data	No Data	No Data
Warrior insecticide cattle ear tag	39039-6	30	No Data	No Data	No Data	No Data

[4] From registrant submitted data to support registration. Compiled by Office of Pesticide Programs Health Effects Division.