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October 22, 2008

VIA EMAIL

Ms. Arthur-Jean Williams
USEPA
Environmental Fate & Effects Division
Office of Pesticide Programs
2777 S. Crystal Drive
South Building – 12th Floor #724
Arlington, VA 22203

Re: October 16, 2008 Meeting on Chlorpyrifos, Diazinon and Malathion Draft BiOp

Dear Ms. Williams:

I am enclosing materials relating to last Thursday's meeting of NMFS, EPA and registrant scientists regarding the referenced draft Biological Opinion, and respectfully request that you have these materials placed in the docket.

The first attachment is a set of detailed notes on the meeting. It was originally prepared by legal counsel who observed the meeting, and has been reviewed by several meeting attendees for accuracy.

Also attached, as referenced in the notes, are the meeting agenda, copies of slides presented at the meeting by registrant scientists, and a summary paper prepared by MANA regarding their slide submission.

Thank you.

Sincerely,

David B. Weinberg

cc: David Menotti
Arlene Pangelinan
Tony Hawkes
Scott Hecht
Pamela Lawrence
Karl Gleaves

Enclosures

bcc: D-C BiOp List

WRFMAIN 12916667.1

Notes on Registrant Meeting with NMFS 10/16/08
Agenda attached—prepared by NMFS after registrants submitted some issues.

10:10 a.m. NMFS lawyer Lawrence opens, notes ACA, asks no effort to reach consensus.

Lawrence, Scott Hecht, Tony Hawke only NMFS personnel present. Attendance list circulated and retained by NMFS.

Hecht: Key to what we are doing is problem formulation. Looking at materials submitted to see if it affects risk hypotheses. Thus identified the questions on the agenda. B is in agenda because we had received from registrants information we had not been aware of re: CURES and other things. Had not seen all the data put together in this format before. Interested in monitoring data, if there is some we are not aware of. The Central Valley data is what we are aware of.

Hecht: We had not previously seen Hall's Orestimba Creek study before you submitted it. Asks Hall to describe it.

First, Hall explains his expertise, then describes the multiyear Orestimba study paper. Notes it shows that high variability of benthic community. This makes it very difficult to tease out relationships re: specific stressors. Hecht asks how often samples were taken, how many replicates, etc. Odenkirchen of EPA asks why its important to use the time periods used. Hall explains protocols, developed by CDF&G, which recommend the Spring period as the preferred sampling period.

Hawke asks how Hall concluded EPT taxa not there due to chemical stress. Hall explains his best professional view that EPT taxa are not common in most CA ag streams. This is likely related to physical habitat limitations such as silt loading etc.

Hecht: Which was reference site? Hall: Did not have one, in traditional sense. Site 10 is above ag activity, but we are working with CA and Reg V to develop. Hecht: That makes it difficult to conclude what are community effects. Hall: Yes, but our time series allows comparisons to be made. It is very difficult to find a reference site in the CV. Chuck Hawkins, CDF&G, and other scientists are working with reference site work group panel to try to find these in CV. They have some in other parts of the state.

Hecht: Data seems to show correlation between OPs and benthic communities. Hall: Yes, but R-squares are very low and relationships are very weak. We have initiated an eight-year analysis of habitat and benthic data for Orestimba Creek and a seven-year analysis for two other San Joaquin Valley streams. In urban streams, with more complete data sets for both chemical measurements and habitat, we see that habitat not chemistry is more important for benthic community health.

Hecht: You used land use as input evidence, not monitoring. Why? Hall: Funding was the primary factor coupled with the actual goal of the study – characterize benthic communities and physical habitat in San Joaquin watershed ag streams. We used township range data, tied to pounds used in plots beside the stream. Odenkirchen: This means it simply may be that ag activity is the factor, not the pesticide – variation is with tillage, not pesticides. Hall: Tillage is fairly consistent on farm plots beside the stream. We have reported high correlations among the three types of pesticides evaluated in our analysis (OPs, pyrethroids and herbicides).

Habic: Do you see any change over time? Hall: Not yet, but hope to be able to do this with eight-year set.

Odenkirchen: How about NAQWA data? Hall: Have looked at it, but hard to correlate. Too spotty.

Odenkirchen: Did you look beyond OPs? Hall: Yes: At pyrethroids and herbicides.

Odenkirchen: What was the data base for the 303(d) listing? Hall: Think it was monitoring data from NAQWA and other data sources, with listing occurring if some particular level is exceeded.

Hecht: Is Coalition doing more monitoring? Hall explains what West Side Coalition is doing. Urges Hecht to talk to Parry Klassen for more detail and to get info on all the other group data.

Hecht: Can you give up more description of sites? What riffles, what pools? Hall: methodology says go to riffles areas if you can. In Orestimba you do not have them. So you go to best available habitat. Hecht: What percentage of sites are pools? Hall: We define best available habitat within the 75 m sampling segment of each stream. Fewer than 25% of those areas have riffles. Hecht: did you find higher diversity there? Hall: No. And, choice of locations made by field biologist with 14 years experience. I will share manuscript on eight years work as soon as possible.

Hecht: What can you tell me about headwaters? Hall: We need water every year, so it is not all that far up stream beyond Route 5. Ultimate headwaters are in mountains. Poletika: But always ephemeral above the point at which Hall samples.

Hecht: Thought very good to try to tie together with land use. Hall: CDPR has best Pesticide Use Information available on this, you should be looking at it.

Hecht: Are there other areas in which people are doing bioassessments? Hall: Jim Harrington and Pete Ode from CDF&G have a large program of bioassessments going on around the state; I will give you their numbers.

Discussion moves to microcosm/mesocosm studies. Hecht: You sent us a lot of information. We went through it. But no assessment of how it applies. I would like to

know what you think – so many systems, etc. Sounds like he is saying they have not been able to review the information enough to draw conclusions of their own.

Schocken: I have some slides on the diazinon mesocosm. I would be happy to review.

Poletika: Let's talk generally first. Our goal is to identify these as an independent line of evidence that "perhaps didn't get enough attention" in draft biop. Are you familiar with these types of studies? Hecht: Yes, and we know huge variation of experimental design. The reports, *e.g.,* Giddings – also say they are cautious in conclusions. So we are interested in knowing how these tie to our hypotheses.

Habic: They give you a feel for what will happen in different systems. Over time, gives you idea of system recovery after pesticide application.

Poletika: Studies done over a period of three decades, studies done for different regulatory programs. First for EPA, more recently for EU. Later ones have different orientation than earlier ones – new analytical and statistical tools, focus on answering specific questions. For chlorpyrifos, the studies show a pretty consistent community level NOEL. Also, because they are model systems they are much richer information sources than other data, even though static not river. Allows you to look at sensitivity of species.

Hecht: What is EPA position on mesocosm studies? Poletika: EPA reserves, not requires (discussion follows on what this means); in Europe, generally required. Poletika: This allows reduction of uncertainty factors.

Odenkirchen: What can you tell about levels of effects in mesocosm studies that can be measured against effects in real world? Poletika: There has been some work. Odenkirchen: But no one really has studied about whether a community level effect in mesocosm study is translatable to effects in the field.

Odenkirchen: Issue I want to know is what weight of evidence is showing this? Poletika: I am not aware of anyone who has looked at that. Hecht: How many mesocosms have fish in them? Poletika: Typically not, because of other effects, but you can tell effects of prey food, etc. Odenkirchen: Is concentration higher or lower than 0.1? Habic: Generally higher. Often, no impact on fish even at 0.5. Odenkirchen: This seems to me to be remarkably consistent with NMFS analysis, so there must be enough data to allow judgment about sustainable resources.

Hecht: We have not gone thru the data to see how this data relates to fish growth. Giddings' study highlighted a paper that had been done with fathead minnows. 0.5 gone in 12 days, down to 0.2 in 2 days. (Frasner and Klein re: chlorpyrifos.) It directly addresses the risk hypothesis NMFS has. With juvenile life stage, it shows reduced growth after a single pulse. I have not back calculated what application rate the pulse equals. But very high on value of this study.

Odenkirchen: We need to come up with a way to make decisions, a benchmark. This may tell us effect on individuals, and we need to understand population effects. Debate within EPA about what these studies say beyond that growth of macroinvertebrates can be affected.

Habic: What would be impact of moving water, drift? Hecht: We would have to develop a study. Hawke: But the chemical quickly dissipated.

Hall: Is there any work on what kind of food preference salmonids have after yolk sac absorbed? Hecht: Opportunistic feeders, different hierarchies for different species. Odenkirchen: Are there size classes? Hecht: Yes. Do you have it? We have some. Have you used it? Not for a correlation.

Schocken: Reviews his slides on diazinon mesocosm. (See attached). Shows lots of food sources. Most sensitive species from 1976 PhD thesis, very likely included a very toxic degradate no longer in product. In a much better more recent study, much higher LC 50. Hall: EPA rejected the 1976 test when it set diazinon WQO.

Schocken responds to many questions. Explains no more sulfotep, so result here is conservative. NMFS people clearly had not previously understood this. Tying this to what is happening now (after all reductions, etc). Schocken notes that almost all monitoring data below 0.1 µg/L, which is well below any direct effect levels, and well below indirect effects on prey.

Odenkirchen: Re: Your 54 NOEC. This consistent with NMFS lambda analysis did not show effect until 50. Hecht: We used slope 4.5. Odenkirchen: But that was the best avail data, and it comes out remarkably close to what the study shows.

Schocken reviews conclusions of study. Monitoring concentrations are much lower than impacts measured in these studies.

NMFS asks for slides. Schocken says of course.

Malathion next. Reiss explains the more haz constituent is (isomalthion) created with heat. Much less in current formulations than historically, in any event. Do not know what was in old product tested in study on which NMFS is relying. We have much better recent data, using formulation in EU, which is slightly different than here, but pertinent. We have given you the studies.

Reiss explains mesocosm and boll weavil eradication program studies. Hawke: Asks what boll weavil rates are? Reiss: Not sure, but there were 24 apps. Hawke says he had not looked at this study yet. Both Hawke and Hecht clearly unfamiliar with boll weavil eradication program. Hecht: Do you work with USDA on this? Yes. Habic explains intense water monitoring part of program, although no "data base" produced.

Poletika turns to SSD data re pertinent taxa. Shows slide re chlorpyrifos exposure sensitivity of particular taxa. Uses geometric mean. Hecht: We maybe need to develop list of taxa that have been identified and relative toxicity and presence of species. Odenkirchen: Do the Services have this data? Answer from Poletika: Yes, we have given them both studies cited and toxitity data from the standard EC risk assessment, normalized to 48 hr per EU requirements.

Lunch

Hecht: We would like to discuss distribution/probability-based lead risk assessments, since we have never done one, and this also brings us into the monitoring data.

Hall: With all probability risk assessments it is critical to do a data driven analysis. With these three insecticides you have a very rich toxicity data set. So you can develop SSDs. Explains Ecofram. NMFS appears unfamiliar with it.

Hecht: How do you select toxicity data?

Hall: Not enough to rely on just pulling numbers from web. Need to go back to the original studies to find if numbers are valid. Low data points may turn out to be wrong. Uses diazinon Gammarus example of error by factor of 10. Hecht: Are there criteria/SOPs (standard operating procedures) that can be used? Hall: Yes. EPA uses them in water programs to develop water quality criteria, has published screening criteria. Odenkirchen confirms. All industry representatives agree.

Hecht: So how do you choose an LC50 where you have got many studies on the same species, *e.g.*, daphnia. Hall: Generally use geometric mean. Depending on what you are trying to do, you may do something else – *e.g.*, use most sensitive stage (larval) information. Odenkirchen agrees.

Hawke: How about the endpoint? You have talked about intraspecies variability. How do you select endpoints used in assessments? Hall: We generally take the measurement endpoints that we have greatest confidence in – survival, growth, and reproduction – because measurements are consistent. Hard to use others, such as "behavior," where methods are variable. Odenkirchen: How do you do temporal? Hall: A couple of ways, you can use geometric means where you have multiple data points or select value from most sensitive life stage if data are available for multiple life stages.

Hawke: How about use of LC 50s, when considering recovery and availability of food for the fish?

Hall: When you have a very robust data set, you can select a centile where you have confidence level – often 10^{th} centile, sometimes 5^{th} centile. What you must have is a consistent measure used across all taxa. The LC50 is the most reliable and consistent number. Poletika: Gesey study did this several different ways – found out 10^{th} centile

SSD pretty much same as using EPA methodology, compared it to mesocosm, which also were pretty much the same – this convergence added confidence in analysis.

Hawke: There may be a possibility to apply SSD to organisms that make up foodstuffs. Lenwood: Suter in his risk assessment book has a good chapter about how to do this with related species (phylogenetic closeness approach). Poletika: Here also are publications about what is the minimum number of data points. Need 8-10-12 species.

Odenkirchen: But you also have to look at the biology, to decide how many points are looked at and draw conclusions. Probability assessments are of concentrations over the entire US, but its very different thing to look at a concentration probability at a particular portion of watershed in a particular part of NW.

Odenkirchen: NAQWA data has geographical linkages; NMFS agrees it has this data. Habic says this data is sufficient to allow application of probability assessment methodology. Poletika: The analyses done to date may not fit your hypothesis perfectly, but the methodology they describe can be used.

Hecht: How to we determine the reliability of the data we have? Hall: You have to start with understanding of exposure profile. With diazinon, you are going to have spikes. Therefore, acute data is appropriate to use. If you were dealing with a chemical with a long half-life, sometimes chronic is used, but the three insecticides we are discussing do not have long half-life. Odenkirchen: EPA does this regularly.

Hawke: Acute exposures do not necessarily limit acute problems. Effects over a longer time may be of concern. Odenkirchen: But if we have mesocosm data, and has many species, and has data on concentrations over time, so you can do just this kind of analysis. You need to look at this data and use it in meaningful analysis, not just take a single number.

Poletika: Heidelberg data very good, allowed typical chlorpryrifos pulse was 48 hrs. I found the same thing in a Oresimba creek study I did. This was a separate study from Hall's. Done in 1996. Hecht: Did you use same assessment locations as Hall? No, some time between the two.

Hawke asks whether they can rely on EPA "core" classification to mean study can be relied upon? Odenkirchen: Absolutely. But now we use the term "acceptable." "Supplemental" simply means there are deviations from guideline, but not sufficient to render study invalid.

Hecht: Where is EPA on using SSD? Odenkirchen: We have several chemicals in analysis in which we are using it. At least one with codistributions of exposure and SSD's aquatically and terrestrially. Probability assessments have been considered in a number, often there are questions of data adequacy. Hecht: Does EPA have guidelines? Odenkirchen: Yes, they are what everyone uses, although they are fairly generic, although there is a group working on more refined.

Hecht: How do you handle uncertainty in the tox data? Odenkirchen: In aquatic side, Oldenberg and Slope (phon) of Netherlands have developed method which EPA uses. Hall: You will never test every species. Where you have only on 8-10 species, this methodology allows you to address this issue. Hawke: What was year of that publication? About 1996. Hawke: Is it in the open literature? Odenkirchen: Yes, plus there is a Netherland government tech paper. SAB also considered issue a few years ago and information will be in their dockets. Habic: In fact, OS probably have a software you can use – simple spreadsheet.

Hawke: How do you address the adequacy of the sampling *vis a vis* the Sacramento and San Joaquin diazinon analysis? Hall: We have used six sources or more, all from different programs with different goals, detection limits, etc. But you have a pretty good spatial and temporal data set and sampling data pertinent to particular species. What I would do is determine the overlap of the salmonid ESU habitat with the stations having monitoring data. I think you will find at least some overlap. Data quality meets DPR standards.

Odenkirchen: What is portability of that data to other areas? Hall: The data is pretty good for our area and uses. Applicability elsewhere will depend on use patterns in that area, water body characteristics.

Odenkirchen: Do you not also have to factor in life stage, areas, etc? Hall: Absolutely. This is very tough job – you have got 28 different ESUs units to look at and need to apply appropriate analysis to each. Odenkirchen: So you cannot just do a single number for all of the NW? Hall: Correct.

Hecht: But even with Central Valley, where you have the best data, you have huge gaps and cannot do many trend analyses, etc. In CV, we have fish living in rivers for a year, in various habitat. Odenkirchen: Does that not mean each watershed is different? Hecht: Generally uniform across species, lots of overlap. We have been digging into life history data we have by population by ESU. Uses one with 22 different habitat. But we have monitoring data only as to one. As to many ESUs, we have great fish count information, but these are outside the Central Valley, but not good pesticide use info. In CV, we have great pesticide use data, less fish information.

Reiss: So how do you see the value of monitoring data v. modeling? Hecht: There is no data set that allows analysis for a particular ESU. Habic: So it is hard to validate models? Hawke: Models have been validated. Brady(?) (EPA) says very much so.

Odenkirchen: EPA's issue here, as we go forward with other products, where we know there is variation among ESUs and species, how do we avoid too broad a brush and jeopardy conclusion? Poletika: That is also how you want your protection program to work.

Schocken: MANA has done something even more with diazinon, explains SWAT model. Validated with data. Hawke: Is that a feasible approach for larger area? Williams: There has been lots of work by USDA on impact of vegetative strips etc.

Brady(?): We have lots of data on the Pacific NW. Odenkirchen: One question, though, is how fine an analysis you need to make jeopardy call – must look to biology of species. Will be greater concern with some areas than others.

Hall: How do you tease out natural variability of salmonid populations versus stressors?

Hecht: Every five years we go through a population analysis. Look at variety of metrics. Come up with a ranking. Two different NMFS regions (CA v. Pac NW) involved here, so some difference in approach. Recommend looking at FERC BiOp for Columbia River. We used a lot of this information in this draft BiOp. Odenkirchen asks why this draft is not as comprehensive, adopt same types of analysis as that one. Hecht: Same in terms of steps. Odenkirchen: It is more than just steps; the standard for making decisions should be the same.

NMFS lawyer Carl Gleaves starts saying they are required to assume jeopardy where there is not enough data. Stops when reminded discussion is of science, not law.

Break. Hall spends 10 minutes with NMFS scientists discussing joint probability curves and other risk assessment methods.

Turn to incident data. Hawke says they got incidents from EPA data base. Also had requested studies from DAS a couple of studies. He was most interested with concentrations associated with field kills, but report said that would be handled by registrant. Poletika: I was surprised by that too. It must be buried in the pre-2002 EPA data, but as to us is lost in the past. Hawke: In the citrus study you had said those were misapplication/overspray, but study said there were several fish kills, but were all of those misapplication/oversprays? Would like that information. Poletika: I will look, but since this was incidental to a terrestrial effects study, it was probably only recorded as per FIFRA § 6(a)(2). Also, keep in mind the difference between there areas, etc. from what you are now looking at.

Hawke: Same thing arises as to other study. Poletika: Which ones? Hawke: I will send you email. Williams: Please cc us.

Reiss: Re: other issues. I want to revisit modeling. Your model numbers come up much higher for malathion than any monitoring data. Talks about the limits of the farm pond model. Brady (?) explains various conservatizing assumptions. Reiss: We do not see how farm pond model relevant here. Hawke: Salmonids use lots of different habitats, we understand limits. Habic: That is why you have to distinguish among specific ESUs and areas. Schoken: SWAT model allows you to make better evaluations about variation. Prism/exams is forced, not the best tool you have. Reiss: There is a USGS WARP model that focuses on chronic. Odenkirchen: Has only been used on atrazine, does not

give you temporal or acutes. EU also uses prism/exams. SWAT to hard to use, to find appropriate segments or watersheds. Very hard to do this. We see prism/exams as best for low order streams.

Menotti: Asks about how you use prism/exams when doing probablistic evaluation. Odenkirchen: We have evaluated, gone to SAP. Sometimes you can find exceedences of predicted results among the field data. But it serves the purpose for which we use it. For sturgeon we evaluated impact of flow. Real question is what tools are best applied to make judgments pertinent to different types of habitat. More rocks have to be turned over.

Turn to point B on agenda. Hecht: We had not seen the information on CURES and BMPs before you filed it. What is going on in other areas? Everich: The CA restrictions you have seen are in place everywhere but two counties. Hecht: Which ones? Everich: Do not know, but I will let you know. But statewide dormant spray regulations apply everywhere. These are not adopted in other states but could be. Discussion. NMFS clearly does not understand supplemental label. At some point, Hawke asks whether a supplemental label exists for chlorpyrifos. Poletika says no, but the dormant spray regulations apply. Discussion returns to Coalitions and CURES, and how widespread their activities are. Information on website. Weinberg speaks up a bit on CA water programs and peer pressure to join up and comply. They seem to have been generally aware of that the ag waiver program existed, but not of any detail or context Hall explains how Coalitions had to file plans; he is on tech committee is advising; Klassen is a source of more information. NMFS asks if the data collected under Coalition plans overlaps with NAQWA water data (revealing they previously haven't looked). Hall says maybe a few. They should call Joe Karkowski of the Water Board.

Hecht asks about Sac River Watershed Program: What have they been doing? Hall explains, suggests they talk with Claus Suverkropp at Larry Walker Assoc. At Odenkirchen's request Hall will cc Williams on any contact info he provides to NMFS.

Hecht: What is the Landguard program? Poletika: Enzyme technology developed in Australia by CSRO. Has been used, but status of company offering it Orica (phon) is uncertain. Vetted by water boards with toxicity texts, etc. Hawke: Why was it a separate tab in material registrants filed? Weinberg says probably just a clerical decision.

Hecht: What do you see as top three ways to reduce impacts on water? Poletika says local decisions control drift. Weinberg points out he is not writing on a blank slate. Hecht says he means in general. Poletika says irrigation system control, but this is not limited just to these three molecules. Vegetative strips very effective. Poletika say CURES and NRCS have lots of programs in place.

Hecht: Can you (Chemi) explain why you said "will be implemented" re: RED. Chemi representatives explain labels still in process. Hawke: Asks question about how label review process works. Is there a firm date? Williams: One will be set.

Hawke: Are buffer strips on labels? Odenkirchen: Some. Williams: Hard to enforce, so we are not fully comfortable putting them on labels. Hecht: Could they be included on a labels? Williams: We could talk about it. But CURES related stuff might be part of baseline.

Everich: What are you intentions.? Hawke: To consider what we have been told.

End at 3:45 p.m.

12916192.2

DRAFT AGENDA

MEETING WITH EPA, NMFS, APPLICANTS MANA, DAS, AND CHEMINOVA
OCTOBER 16, 2008
9:30 am – 5:00 pm
12:00-1:00 lunch on your own

SSMC 2, Rm 16246
NMFS
Silver Spring, MD

Meeting Objective: Opportunity for NMFS to seek clarification and ask questions on applicant-provided materials

Meeting will be facilitated by NMFS

A. Applicability of information to risk hypotheses and assessment endpoints:

1. Studies on field measurements of biological communities and effects to salmonid prey items

2. Interpretation of mesocosm data to threatened and endangered salmonids and habitat

3. Interpretation of past ecological risk assessments

4. Areas of uncertainty identified in applicant-provided information

5. Representativeness of Central Valley California studies to other ESUs

6. Incident information

7. Other Issues

B. Current risk mitigation measures to minimize the effects of the active ingredients on listed Pacific salmonids:

Identification, implementation, and effectiveness of current Best Management Practices (BMP) for chlorpyrifos, diazinon, and malathion uses

1. Drift

2. Runoff

Diazinon Microcosm and Mesocosm Studies

Summary of Results and Interpretation

Microcosm Study - Giddings et al., 1996
(Effects of Diazinon on Large Outdoor Pond Microcosms, Environ. Toxicol. Chem., 15(5), pp 618 – 629, 1996

- Study Design:
  - Outdoor microcosm study in tanks 3.2 m diameter x 1.5 m deep. Eight treatment levels (nominal 2 – 500 µg/L; measured 2.4 – 443 µg/L) with treatments applied three times at seven-day intervals; test system contained natural water + sediment; bluegill sunfish were fish species; study lasted 4.5 months
- Results:
  - Cladocerans were the zooplankton group most severely affected (*e.g., Daphnia and Ceriodaphnia* spp.). Diazinon significantly reduced cladoceran abundance at all treatment levels throughout the study.
  - Other zooplankton, namely, copepods and rotifers , were affected by diazinon **but recovered**.
  - All macroinvertebrates (except Ephemeroptera (e.g., mayflies) at the highest treatment level) recovered by the end of the study.
  - Fish survival was diminished at 54 µg/L and higher concentrations. Fish biomass affected at 22 µg/L.
- Discussion:
  - Overall NOEC occurred at 4.3 µg/L. Although cladocerans were practically eliminated, they were **not** the major components of the zooplankton community. Other taxa, although initially affected, recovered over time.

Microcosm Study - Giddings et al., 1996
(Effects of Diazinon on Large Outdoor Pond Microcosms, Environ. Toxicol. Chem., 15(5), pp 618 – 629, 1996

- Discussion - continued:
  - LOEC considered 9.2 µg/L. Although there were effects on all three major zooplankton groups and on macroinvertebrates, all groups recovered within six weeks of the last diazinon application (except cladocerans).
  - Curiously, whereas the midge, *Chironomus tentans* had a reported 96-hour LC50 of 0.03 µg/L the LOEC for Chironomini (the tribe to which *C. tentans* belongs), was 54 µg/L, **more than three orders of magnitude higher than the reported LC50 of the single species.** Thus, the laboratory data greatly overestimated the sensitivity of the tribe Chironomini . (**Importantly, note that the *C. tentans* laboratory test result was likely confounded by the presence of sulfotepp, a very highly toxic degradate/impurity that was present in technical diazinon and diazinon formulations at that time. Subsequently, formulations were stabilized to prevent the formation of sulfotepp and therefore, great caution should be exercised in interpreting older diazinon toxicity testing results.**)

Microcosm Study - Giddings et al., 1996 (Effects of Diazinon on Large Outdoor Pond Microcosms, Environ. Toxicol. Chem., 15(5), pp 618 – 629, 1996

- Significance of Results:
  - Bluegill sunfish have similar 96-hour LC50s (136 – 460 µg/L) to salmonids (rainbow trout [90 – 400 µg/L]); bluegill survival not affected (based on statistical analysis with controls) until diazinon exposure reached 54 µg/L in microcosm study.
  - Based on recent surface water monitoring results, diazinon concentrations are much lower than concentrations that would affect salmonids directly (survival) or indirectly (effects on their prey because at environmentally relevant concentrations, many prey species are not impacted by diazinon).

Mesocosm Study – Giddings, 1992
(Aquatic Mesocosm Test for Environmental Fate and Ecological Effects of Diazinon, Springborn Laboratories, Inc., MRID 42536901)

- Study Design:
  - Mesocosms were replicated 0.1-acre ponds containing sediment and water, stocked with bluegill sunfish. Five diazinon treatment levels + controls. Ponds treated six times, alternating between spray and direct aqueous applications. Treatment regimes created 96-hour maximum concentrations ranging from 2.3 – 28 µg/L. Study lasted five months.
- Results:
  - Cladocerans severely reduced at all diazinon treatment levels. Copepods and rotifers were less sensitive, with effects first occurring at exposure levels in the range of 14 – 28 µg/L. Among the insects, caddisflies and some midges were affected at the lowest levels (2.3 µg/L). Mayflies and several groups of midges were affected at 8.4 – 28 µg/L. Damselflies were affected at 14 µg/L. **Bluegill sunfish showed no effects for individual growth, reproduction and survival at any treatment level.**
- Discussion:
  - Although some effects occurred at the lowest treatment levels, they were confined to cladocerans and numerically minor insect taxa and did not alter the overall structure or function of the ecosystem.

Mesocosm Study – Giddings, 1992
(Aquatic Mesocosm Test for Environmental Fate and Ecological Effects of Diazinon, Springborn Laboratories, Inc., MRID 42536901)

- Discussion - continued:
  - The lowest adverse ecological effects level (LOEC; effects on major invertebrate groups) occurred at the 8.4 µg/L treatment level. As written by EPA in the recent California Red-legged Frog risk assessment, "*The overall impact of diazinon on the aquatic community was that many aquatic invertebrates were affected at treatment concentrations greater than 11 µg/L;* ***however, most taxa recovered after treatment****. Although significant reductions were observed in macroinvertebrate abundance throughout the study period, fish and plants were generally unaffected by the diazinon treatments. Under the study conditions, mesocosms treated with multiple applications of diazinon* ***did not reveal any statistically significant direct or indirect effects on fish even though there were significant fluctuations in aquatic invertebrates due to diazinon.*** (emphasis added)"
- Significance of Results:
  - Results of a complex study that incorporates aggregate responses of multiple species to diazinon showed that fish are <u>not</u> adversely affected at concentrations as high as 28 µg/L. Based on recent surface water monitoring, diazinon concentrations are much lower than those that would affect salmonids directly (survival) or indirectly (effects on their prey given their widely different sensitivities and their ability to recover from exposures).

Composition of Prey (i.e., Sources of Food) for Pacific Salmonids and the Impact of Diazinon

Listing of Food Sources for Pacific Salmonids

Food Sources	Representatives	LC50 Ranges
Cladocerans	*Daphnia, Ceriodaphnia, Bosmina*	0.32 – 10 µg/L
Copepods	*Heterocope, Diaptomus, Epischura, Cyclops*	2.6 – 2,510 µg/L
Amphipods	*Grammarus, Hyallela*	2 – 200 µg/L
Caddisflies	*Hydropsyche recurvata*	220 µg/L
Mayflies (nymphs)	*Baetis intermedius*	24 µg/L
Damselflies	*Lestes congener*	50 µg/L
Dragonflies	*Orthetrum albistylum*	140 µg/L
Stoneflies (nymphs and adults)	*Pteronarcys californica, Acroneuria ruralis*	16 -25 µg/L
Midges (larvae, pupae and adults)	*Chironomus tentans*	0.03* – 10.7 µg/L
Fish larvae	*Osmeridae (smelts)*	Not Available
Juvenile salmon	*Oncorhynchus*	90 µg/L

*Result highly suspect because study done in 1976, before existence of GLP regulations and independent auditing; exposure concentrations not measured: test material a 50% diazinon-containing formulation that likely also contained the impurity/degradate sulfotepp which is ~10-fold more toxic than diazinon to daphnids and, since the 1980s, diazinon formulations contain a stabilizer to prevent formation of sulfotepp. In addition to this 1976 study, another more recent study indicates *C. tentans* to have an LC50 of 10.7 µg/L, more consistent with results seen in Giddings' microcosm study.

Food Sources for Juvenile Pacific Salmonids - Conclusions -

- Prey items of juvenile Pacific salmonids are varied and comprised of mayflies, caddisflies, stoneflies, damselflies, dragonflies, midges, amphipods, cladocerans, copepods, fish larvae and other juvenile salmonids.
- The range of diazinon sensitivity is very broad, from 0.32 µg/L (*Ceriodaphnia dubia*) to 2,510 µg/L (*Cyclops sp*., a copepod).
- Although certain potential components of the salmonid diet could be impacted by diazinon, alternative food sources with much less sensitivity to diazinon would be available.
- At levels of diazinon currently found in surface water, any adverse impact on potential salmonid food sources, collectively, should be minimal.

Summary of MANA Presentation Re: Prey Items and Microsoxm/mesocosm studies
At Oct 2 meeting with NMFS

Prepared by Dr. Mark Shocken

MANA presented two sets of slides that covered prey items of Pacific salmonids and a summary of diazinon microcosm and mesocosms studies conducted by Jeff Giddings and his colleagues in the early 1990s.

The first presentation showed a table listing food sources of juvenile Pacific salmonids. Prey included cladocerans, copepods, amphipods, caddisflies, mayflies, damselflies, dragonflies, stoneflies, midges, fish larvae and juvenile salmon. Toxicity benchmarks (LC50s from single-species toxicity testing) ranged from 0.32 μg/L (cladoceran) to 2,510 μg/L (copepod). MANA pointed out that the most sensitive prey item, a *Chironomus tentans* midge, had been thought to have an acute LC50 of 0.03 μg/L which made it extremely sensitive to diazinon. However, upon further review using the EPA's ECOTOX database, it was found that the study came from a 1976 Ph. D. dissertation where the actual test material was a 50% diazinon-containing formulation that very likely contained a diazinon degradate/impurity known as sulfotepp. Sulfotepp is more toxic than diazinon and, against daphnids (cladocerans), has an LC50 ten times lower than diazinon. Subsequently, however, sulfotepp has been removed from diazinon formulations by using stabilizers such as epoxidized soybean oil (EPO). Importantly, EPA has cautioned against using older data such as this one because of the very likely presence of sulfotepp in both technical diazinon and in diazinon formulations. California has also acknowledged this problem with older diazinon toxicity testing. In another, more recent *Chironomus tentans* toxicity test result obtained from the ECOTOX database, the LC50 was determined to be 10.7 μg/L or 357 times less toxic than the 1976 study.

In summary, prey items of juvenile Pacific salmonids are varied and the range of diazinon sensitivity is very broad. Although certain potential components of the salmonid diet could be impacted by diazinon, alternative food sources with much less sensitivity to diazinon would be available. At diazinon levels currently found in recent surface water monitoring (generally less than 0.1 μg/L), any adverse impact on potential food sources, collectively, should be minimal.

MANA's second presentation summarized and interpreted the results of diazinon microcosm and mesocosm studies. Both studies were conducted by Giddings et al. The microcosm study showed that cladocerans were the zooplankton group most severely affected. Other zooplankton, namely copepods and rotifers, were affected by diazinon but recovered before the end of the study. The same was true for the

macroinvertebrates except the mayflies at the highest treatment level (443 μg/L). Fish survival was diminished at 54 μg/L and higher based on statistical analysis between treated microcosms and controls. The overall NOEC was considered to occur at 4.3 μg/L. Although cladocerans were practically eliminated, they were not the major components of the zooplankton community. Other taxa, although initially affected, recovered over time. The LOEC was considered 9.2 μg/L. Although there were effects on all three major zooplankton groups and on macroinvertebrates, all groups recovered within six weeks of the last diazinon application (except cladocerans).

Interestingly, the LOEC for the tribe Chironomini (the tribe to which *C. tentans* belongs) had a LOEC of 54 μg/L which was more consistent with results of single-species toxicity testing based on the more recent study rather than the 1976 study that likely contained the toxic impurity sulfotepp.

The mesocosm study showed similar results to the microcosm study. Cladocerans were severely reduced at all diazinon treatment levels and did not recover. However, copepods and rotifers were less sensitive with effects first occurring in the range of 14 – 28 μg/L. Insects such as caddisflies, mayflies and midges were also not affected until exposure concentrations were higher (8.4 – 28 μg/L). Importantly, bluegill sunfish showed no effects for individual growth, reproduction and survival at any treatment level (between 2.3 and 28 μg/L). The LOEC was considered 8.4 μg/L, similar to the results from the microcosm study. EPA, in considering the results from this study, noted that fish were generally unaffected by the diazinon treatments, either directly or through impacts on their prey.

Results from both the microcosm and mesocosm studies showed that the concentrations of diazinon required to impact fish such as salmonids is much higher than results of recent surface water monitoring. That is, the vast majority of surface water monitoring since 2004 show diazinon at concentrations less than 0.1 μg/L, much less than the NOECs obtained from the microcosm and mesocosm studies.

Furthermore, as Ed Odenkirshen pointed out, results of the NMFS population modeling showed that effects on juvenile salmonids from diazinon were not significant until exposure concentrations reached around 50 μg/L which is in line with results from the microcosm/mesocosm studies.

MANA also suggested that EPA and NMFS consider the use of the SWAT model, a watershed-scale model that has previously been used to predict diazinon surface water concentrations in the Feather River watershed in Northern California. The model also was able to evaluate the benefits of various best management practices such as a vegetated filter strip, a no-spray buffer zone and not applying pesticide 72 hours

before a significant rain event. A model such as SWAT was promoted as a potential improvement over edge-of-the-field models such as PRZM-EXAMS and GENEEC for salmonid habitats. Both EPA and NMFS appreciated the possible utility of SWAT and another model (WARP) but commented that these models are specific to particular watersheds and it would be difficult to use them over the entire salmonid habitats of the Pacific states. Nonetheless, MANA pointed out that it might be relevant to use them in the most vulnerable salmonid areas.