Appendix G
Information on Multiple Active Ingredient Products

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[1] [2].

Acute oral toxicity data (i.e., LD50 values) from mammalian studies for formulated products that contain metolachlor and one or more additional active ingredients are summarized below.

Currently, the Agency's guidance for assessing the potential risk of chemical mixtures is limited to human health applications (USEPA, 2000). However, the guidance includes principles for evaluating mixtures to assess potential interactive effects that are generally applicable. Consistent with EPA's Overview Document (USEPA 2004), the Agency's mixture guidance (USEPA 2000) discusses limitations in quantifying the risk of specified mixtures when there is differential degradation, transport and fate of chemical components following environmental release or application. The LD_{50} values are potentially useful only to the extent that a wild mammal would consume plants or animals immediately after these dietary items were directly sprayed by the product. Increasing time post application, the differential rates of degradation, transport, etc. for the active ingredients in the formulation only permit a qualitative discussion of potential acute risk (USEPA 2004).

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 LD_{50}s, with associated 95% confidence intervals, are needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that many of the formulated products do not have LD50 values of the required quality and since LD_{50} values are not available for all the components of these formulations a quantitative analysis of potential interactive effects is not possible.

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

[2] 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).

While a quantitative evaluation of the data is not possible with currently accepted scientific methods, as a screening tool, a qualitative analysis can be used to indicate if formulated products exhibit interactive effects (e.g., synergism or antagonism). In the case of metolachlor, a qualitative examination of the trends in LD50 values, with the associated confidence intervals, across the range of percent active ingredient, show no discernable trends in potency that would suggest synergistic (i.e., more than additive) or antagonistic (i.e., less than additive) interactions. In addition, when the product LD50s, and associated confidence intervals, are adjusted for the percent metolachlor (a conservative assumption that attributes all of the observed toxicity of the formulated product to metolachlor) in 4 out of the 9 cases these adjusted 95% confidence intervals overlap with the confidence values of the LD50 value of metolachlor. In the other instances the adjusted LD50s and/or the confidence intervals are within a factor of 2; given the overall variability of the available acute toxicity data these differences are not considered biologically significant. Based on this qualitative evaluation of the best available data and the Agency's existing guidance it is reasonable to conclude that these formulations are reflecting an independent additive toxicity response and not an interactive effect. Given that the active and inert ingredients would not be expected to have similar mechanisms of action, metabolites or toxicokinetic behavior it is also reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment of metolachor's potential effect on the CRLF when it is co-formulated with other active ingredients can be based on the toxicity of metolachlor.

Pesticide Products Formulated with Metolachlor and Other Pesticide Active Ingredients

METOLACHLOR PRODUCTS [i] [ii]

PRODUCT/TRADE NAME	EPA Reg.No.	% Metalchlor	PRODUCT		ADJUSTED FOR ACTIVE INGREDIENT	
			LD50 (mg/kg)	CI (mg/kg)	LD50 (mg/kg)	CI (mg/kg)
Drexel trizmet ii	19713-547	26.1	>=2000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Metolachlor at	19713-593	34.8	2000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Triangle Herbicide	66222-131	34.5	>=2000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Parallel Plus	66222-132	28.9	>=2000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Bicep II Magnum	100-817	26.1	3271	2755-3882	854	719-1013
Bicep Lite II Magnum	100-827	35.8	4824	3660-6358	1727	1310 -2276
Bicep Magnum	100-886	26.1	4294	3284-5615	1121	857-1466
Boundary Herbicide	100-958	68.1	2586	2305-2900	1761	1570-1975
Camix Selective	100-1148	36.8	>5000	NA Limit Dose	NA Limit Dose	NA Limit Dose

Lumax Selective	100-1152	29.4	2865	No Data (ND)	ND	ND
Expert Herbicide	100-1161	18.6	>=2000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Boundary 6.5EC	100-1162	58.2	1805	1444-2339	1051	840-1361
Brawn herbicide	100-1165	26.1	ND	ND	ND	ND
Sequence Herbicide	100-1185	29	>5000	NA Limit Dose	NA Limit Dose	NA Limit Dose
Newconcept Herbicide	100-1201	19	4144	1485-20,000	787	282-3800
Bicep Lite II Magnum	100-1213	35.8	4824	3660-6358	1727	1310-2276
Bicep II Magnum	100-1214	26.1	3271	2755-3882	854	719-1013
Prefix herbicide	100-1268	46.4	5000	2865-8390	2320	1329-3893
Dupont Cinch ATZ Lite	352-623	35.8	ND	ND	ND	ND
Dupont Cinch ATZ	352-624	26.1	ND	ND	ND	ND
Charger Max ATZ	1381-199	0.7	ND	ND	ND	ND
Charger Max ATZ Lite	1381-208	35.8	ND	ND	ND	ND
Stalwart extra	60063-23	26.1	>=2000	NA Limit Dose	NA Limit Dose	NA Limit Dose

Reviews of Open Literature Studies on Formulations and Multiple AI Products

Chemical Name: Atrazine (Impact of mixtures on Leopard frog and African clawed frog tadpole; lab study)

ECOTOX Reference Number and Citation: 85815

Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: Are We Understanding the Impact? Env. Health Persp. 114 (1). p. 40-50.

Date of Assessment: 9/25/06

Brief Summary of Study Findings:

9 pesticides (4 herbicides: atrazine, metolachlor, alchlor, and nicosulfuron; 3 insecticides: cyfluthrin, cyhalothrin, and tebupirimphos; and 2 fungicides: methalaxyl and propiconizole) were assessed individually (0.1 ppb) and in 3 different mixtures (atrazine + S-metolachlor at 0.1 and 10 ppb, Bicep at 0.1 and 10 ppb, and 0.1 ppb x mix and 10 ppb x mix) to determine effects of realistic pesticide mixtures typically applied to Nebraska cornfields. In addition, atrazine and S-metolachlor combined (0.1 or 10 ppb each) and the commercial formulation of Bicep II Magnum (which contains both of these herbicides) were examined. Endpoints in the leopard frog (*Rana pipiens*) included larval growth and development (i.e., time to foreleg emergence [FLE], time to complete tail resorption [TR], snout-vent length (SVL) and body weight [BW] at metamorphosis), mortality, gonadal development (i.e., sex differentiation), thymus histology and disease rates (i.e., immune function), and the interaction between time to TR and SVL and BW at metamorphosis. The effects of the 9-compound mixture on plasma corticosterone levels were also examined in male African clawed frogs (*Xenopus laevis*).

Methods for effects of pesticides on larval Leopard frogs: Atrazine = $\geq$ 98% ai; Bicep II Magnum reported as 33.3% atrazine, 0.7% atrazine-related products, 26.1% TGAI of S-metolachlor (18.57% S-met. And 2.5% R-met.), and 40.2% inert ingredients). Larvae (30/tank) were reared in 4 L of aerated 0.1x Holtfreter's solution and fed Purina rabbit chow. Tanks (plastic mouse boxes – size unspecified) were covered throughout the experiment and food levels were adjusted (not specified) as larvae grew to maximize growth. Experiments were conducted at 22-23^{o} C with 12/12-hr light/dark cycle. Larvae were treated by immersion with 0.1 pbb of each pesticide. A mixture of all 9 pesticides was tested at 0.1 ppb each and 10 ppb each; in addition, a 2-compound mixture (atrazine + S-met.) was tested at 0.1 and 10 pbb, and a commercial prep of Bicep II Magnum was also tested. Comparisons between Bicep and the atrazine + S-met mixture were examined to estimate the potential effects of the solvent used in the Bicep mixture.

Ethanol was used as a solvent for all pesticide treatments and was included in the control (no negative control was included). Each treatment was replicated 3 times (30 larvae/rep). Cages were cleaned, water changed, and treatment renewed every 3 days. Exposure period lasted throughout the larval period from 2 days post-hatch until

complete tail resorption (TR; Gosner stage 46). Nominal concentrations were confirmed via lab analysis. Atrazine was detected at 0.19 ppb.

Histological analysis of the gonads and the thymus (to measure immunocompetence) was completed. Thymus histology was completed after noting that animals exposed to pesticide mixture experienced increased disease rates due to pathogen *Chryseobacterium (Flavobacterium) menigosepticum*. Effects of single pesticides (20 animals each), the 0.1 ppb Bicep mixture, and the 9-compound mixture were examined.

Methods for effects of pesticide mixture on corticosterone levels in adult African clawed frogs

The effects of the 9-compound pesticide mixture on plasma corticosterone levels was examined used adult male *Xenopus laevis* (used as surrogate because metamorphic leopard frogs are too small to obtain repeated blood samples and because *X. laevis* are available year-round). Five males were treated with the 9-compound pesticide mixture (including atrazine at 0.1 ppb) and five males were exposed to ethanol only (no negative control was tested). During the 27 day exposure period, no aeration was provided, the animals were fed Purina trout chow daily, and solutions were changed and treatments renewed every three days. Blood was collected by cardiac puncture.

Results (specific for atrazine only)
No single pesticide affected mortality or time to metamorphosis ($p > 0.05$). Animals exposed to pesticide mixtures at 0.1 ppb had significantly longer larval periods – initiation of metamorphosis (days to FLE and TR) was delayed. Size at metamorphosis (SVL and BW) was significantly less in the 0.1 ppb atrazine treatment than the ethanol control ($p < 0.05$); however, no negative control was tested. All mixtures resulted in reduced growth (BW and SVL), with the atrazine + S-met. mixture having the greatest negative effect.

With respect to gonadal development, the gonads and gametes were underdeveloped (i.e., gonadal development was delayed) in both the control and treatment groups; therefore, it was not possible to assess the affects of atrazine or mixtures on sex differentiation.

Animals exposed to the 9-compound mixture contracted flavobacterial meningitis; the condition of the thymus was examined as an estimate of immune function. Exposure to atrazine and S-met. resulted in damage to the thymus as measured by thymic plaques. No other single pesticide produced this effect. The 9-cmpd mixture also had a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males ($p < 0.05$).

Discussion

The authors indicate that realistic pesticide mixtures at ecologically relevant concentrations have effects on amphibian development and growth, which may lead to survivorship impacts. Hayes et al. also contend that the study confirms the retardation of

amphibian development for atrazine, and also shows a reversal of the relationship between time to metamorphosis and size at metamorphosis.

It should be noted that the study authors were unable to replicate the results of previous experimental findings of ovarian tissues in testes, instead attributing the disparate findings [on atrazine gonadal development effects] to population variability. The effects of atrazine on the gonads were not detectable because individuals from the population of animals used in the experiment did not complete sexual differentiation of the gonads before metamorphosis. The author attributes these disparate findings regarding the effects of atrazine on gonadal development to population variability.

Impacts to growth and development were more severe when atrazine was combined with other pesticides (S-metolachlor) and the 9-compound mixture had the most severe impact. The authors suggest that the delay in metamorphosis, along with reduced size, will reduce adult recruitment and the likelihood of reproduction. According to the authors, smaller size may limit food availability, increase susceptibility by predators (i.e., be less likely to find food and more likely to be preyed upon), decrease the chances that amphibians will survive overwintering, delay reproductive maturity, and decrease fecundity.

Exposure to the Bicep mixture (atrazine and S-metolachlor) and the 9-compound mixture resulted in damage to the thymus as measured by thymic plaques; however, the ecological relevance of thymic plaques is not discussed. Given the increased incidence of disease and evidence of histological effects on the thymus in animals exposed to the mixtures, the study authors suggest that exposure to pesticide mixtures renders amphibians more susceptible to disease as a result of immunosuppression.

Description of Use in Document: Qualitative use in ESAs.

Rationale for Use: Used qualitatively.

Limitations of the Study: In addition to the lack of negative control testing and the inability of the study authors to replicate the results of previous experiments showing impacts to gonadal development, the following additional limitations were observed in the study design and reporting of data: no raw data or water quality data were provided; the use of plastic test containers that may leach varying amounts of plasticizers, feeding rates, and the quality of food were not described; and only one exposure concentration was tested for the individual pesticides. In addition, the study author's use of "open" literature to support the contention that atrazine affects time to metamorphosis and weight at metamorphosis is misleading. The work by Carr et al. (2003), supposedly substantiating the effects, was previously demonstrated to be a result of inadequate husbandry. In the Carr et al. (2003) study, the animals were starving and were exposed to poor environmental conditions; thus, the larvae's physical resources were likely focused on survival, rather than growth and development.

The study authors report a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males. However, there are several flaws in the study design that add a high degree of uncertainty to the results. First, water quality parameters, including ammonia (which could be a major source of stress) were not measured as part of this study. Secondly, only one single treatment concentration was tested; therefore, it is unclear if there is a dose response. Thirdly, the study author's fail to mention whether the animals were housed in one or separate tanks. If the animals were housed in one tank, the treatment unit would be the tank. Most importantly, the collection of repeated blood samples via cardiac puncture is likely to cause severe trauma to the animals; therefore, the study conditions are conducive to elevating the very endpoint the researchers are attempting to measure (i.e., elevation of blood corticosterone). In summary, many of the confounding effects identified in previous studies by the FIFRA SAP limit the utility of this study.

Reviewer: Anita Pease, ERB3

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

[ii] Metolachlor: LD50= 2514 mg/kg; CI= 2084 to 3126 mg/kg.