CENTER FOR FOOD SAFETY

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RE: Docket EPA-HQ-OPP-2016-0385

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Center for Food Safety appreciates the opportunity to submit supplemental comments for the Scientific Advisory Panel's consideration. In these supplemental comments, we compare EPA's evaluation of glyphosate's carcinogenic potential to its evaluations of two other pesticides that are likely to be carcinogenic to humans, and find substantial differences in assessment standards. In-text references are listed at the end, and are also being uploaded to the docket under filenames that match the in-text references (e.g. EPA CPRC Isoxaflutole 1997, EPA CARC Iprovalicarb 2002).

Our first set of comments addressed EPA's evaluation of glyphosate's carcinogenic potential with reference to EPA guidelines on the conduct and assessment of carcinogenicity studies (EPA 1998a, 1998b, 2005). These prior comments together with 44 supporting materials were submitted to the docket on 10/13 and posted on 10/25 (EPA-HQ-OPP-2016-0385-0438, tracking number 1k0-8sfg-2uef). However, EPA docket staff changed the names of the supporting materials such that they no longer match the in-text references in the main comment file, making it difficult for readers to selectively consult certain references for points of interest. Therefore, we recommend that those wishing to consult our prior comments and properly named supporting materials do so at our website: http://www.centerforfoodsafety.org/reports/4537/cfs-comments-to-epa-science-advisory-panel-on-the-carcinogenicity-of-glyphosate. These supplemental comments and supporting materials will also be posted to this website.

SUMMARY

As discussed in prior comments, EPA's evaluation of glyphosate's carcinogenic potential violated its guidelines for carcinogen risk assessment in numerous respects. In these supplemental comments, I illustrate these deviations by comparing EPA's treatment of two other pesticides with that of glyphosate.

EPA classified isoxaflutole (an herbicide) and iprovalicarb (a fungicide) as "likely to be carcinogenic to humans." While EPA discounted tumor findings in rodents fed glyphosate at levels approaching or exceeding 1,000 kg/mg bw/day, EPA found both isoxaflutole (ISOX) and iprovalicarb (IPRO) to be likely human carcinogens based primarily on tumor findings at doses ≥ 1,000 mg/kg bw/day. With glyphosate, EPA was concerned exclusively with the potential for dosing to be excessive, and thus improperly included in its weight of the evidence evaluation several negative studies with inadequate dosing. In its evaluations of ISOX and IPRO, EPA showed equal concern that dosing be adequately high to provide a sufficiently stringent test of carcinogenicity as well as not excessive.

For its assessment of glyphosate, EPA introduced the novel toxicological principle that doses fed to experimental rodents should somehow correspond to or approximate anticipated human exposure levels, or at least that tumor findings at doses exceeding human exposure could be safely discounted. In contrast, EPA's assessments of ISOX and IPRO make no reference to this unprecedented approach, which violates EPA guidelines. EPA frequently demanded monotonic dose-response as a criterion of significance for glyphosate (discounting statistically significant trends that were not monotonic), but applied no such standard in assessing ISOX or IPRO. EPA classified ISOX as likely to be carcinogenic to humans on the basis of two of two rodent studies with treatment-related tumor findings. IPRO was classified as likely carcinogenic on the basis of one positive rat study and one negative mouse study. Properly interpreted, at least four of seven rat and five of five mouse studies on glyphosate provide evidence of its carcinogenicity.

While EPA found limited and IARC more extensive mechanistic evidence for glyphosate's carcinogenic potential, EPA classified isoxaflutole and iprovalicarb as likely to be carcinogenic despite negative results in four of four (ISOX) and six of six (IPRO) mutagenicity assays. This is proper practice based on the primacy of animal and human over mechanistic evidence, given our still very incomplete understanding of mechanisms of carcinogenicity. Finally, the inability to definitively determine a carcinogenic mode of action (negative mutagenicity assays) led EPA to apply a linear, low-dose extrapolation approach to determine the carcinogenic potency of ISOX (for liver tumors) and IPRO, an approach that would seem to be justified for glyphosate as well.

EPA's evaluation of glyphosate's carcinogenic potential not only violates its relevant guidelines in numerous respects, it is also at odds with the guideline-compliant approach the Agency applied in assessing the carcinogenicity of two other pesticides that are likely human carcinogens: isoxaflutole and iprovalicarb. The SAP is urged to assess glyphosate's carcinogenic potential in line with EPA guidelines and accepted standards of toxicology.

COMMENTS

In prior comments (accessible at http://www.centerforfoodsafety.org/reports/4537/cfs-comments-to-epa-science-advisory-panel-on-the-carcinogenicity-of-glyphosate), it was shown that EPA's evaluation of the animal evidence for glyphosate blatantly violated Agency guidelines for the conduct and interpretation of carcinogenicity feeding studies. In these supplemental comments, I first summarize EPA's deviation from its guidelines in several areas, and then describe how EPA's treatment of glyphosate also deviates from past practice, as exemplified by the Agency's carcinogenicity assessments of two pesticides: isoxaflutole and iprovalicarb.

1.0 Assessment of Dosing: Is it Adequate and/or Excessive?

1.1 Deviations from EPA guidelines[1]

EPA maintains that its guidelines prescribe a maximum or "limit dose" of 1,000 mg/kg bw/day for use in rodent feeding studies, but this is incorrect. EPA guidelines state that the high dose "should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors," but that it "***need not exceed*** 1,000 mg/kg bw/day" (EPA 1998a, 1998b). The high dose is not defined by some arbitrary number, but rather by the biological effects of the test compound on the test animal. It should be high enough to be toxic, but not so high as to adversely affect survival due to effects other than tumors. The "need not exceed" clause is secondary, and merely ***allows*** (not requires) the performing lab to utilize a high dose ***as low as*** 1,000 mg/kg bw/day when doses exceeding this level are found ***not*** to elicit the otherwise requisite "signs of toxicity." The only upper limit for dietary studies is a practical one: 5% of the test substance in the feed (EPA 2005, p. 2-17), and glyphosate was not administered at this high level in any of the studies reviewed by EPA. EPA also improperly includes at least four negative

[1] For a fuller and documented discussion of this topic, see Center for Food Safety's prior comments to the SAP at http://www.centerforfoodsafety.org/reports/4537/cfs-comments-to-epa-science-advisory-panel-on-the-carcinogenicity-of-glyphosate (Section 2, pp. 9-12).

studies that are not adequately dosed in its weight of the evidence evaluation (Burnett et al. 1979, Pavkov and Wyand 1987, Reyna and Gordon 1973, Suresh 1996),[2] contrary to its guidelines.

Based on misapplication of its own guidelines, EPA improperly discounts the significance of tumors in animals fed glyphosate at doses approaching or exceeding 1,000 mg/kg bw/day; and improperly includes under-dosed negative studies in its weight of the evidence evaluation. The SAP should give full weight to tumor findings at high doses that do not substantially alter the normal lifespan of the animal due to effects other than tumors; and discount the absence of treatment-related tumors in studies that do not employ adequately high doses.

1.2 Deviations from EPA's past practice

1.2.1 Isoxaflutole

In 1997, EPA evaluated two carcinogenicity studies on the herbicide isoxaflutole (one mouse, one rat), and concluded that isoxaflutole was "likely to be carcinogenic to humans" (for the discussion of isoxaflutole throughout these comments, refer to EPA CRPC Isoxaflutole 1997).

In the 78-week CD-1 mouse study, there were statistically significant trends and pairwise comparisons (high-dose vs. control) for liver tumors in male and female mice. The statistically significant trends in both sexes were driven entirely by findings in the high dose groups, in which the animals were fed 977.3/1161.1 mg/kg bw/day (M/F) of isoxaflutole. EPA did not discount findings at this dose because it approached/exceeded 1,000 mg/kg bw/day, as it did with glyphosate. Instead, EPA followed its guidelines by assessing biological effects. The high dose was judged to be adequately high because signs of spleen and liver toxicity as well as decreased body weight gain were observed. The high dose was not excessive because survival was not decreased relative to controls.

In a 104-week study on Sprague-Dawley rats, rats were fed 0.5, 2, 20 or 500 mg/kg bw/day isoxaflutole. Treatment-related liver (male and female) and thyroid (male) tumor findings were largely concentrated in the high-dose groups. EPA noted a broad range of toxic effects in the high-dose animals, and regarded these effects as evidence that "the 500 mg/kg/day dose level is … an adequate dose for assessing the carcinogenic potential of [isoxaflutole] in rats." The dose was not regarded as excessively toxic because there was no adverse effect on survival.

These were the only two carcinogenicity studies evaluated by EPA. Both were assessed for dosing, and both were found to employ adequately high, but not excessive, doses based on EPA

[2] As discussed in Center for Food Safety's prior comments, there are other, even more compelling reasons that the SAP should exclude the first three of these four studies from its evaluation.

guidelines. The EPA reviewers made no suggestion that the dosage levels for the high dose or other treatment groups should somehow correspond to anticipated levels of human exposure, nor that high-dose findings should be discounted because humans are (presumably) not exposed to such high levels of the pesticide, as the Agency did in its glyphosate evaluation.

1.2.2 Iprovalicarb

In 2002, EPA evaluated two carcinogenicity studies on the fungicide iprovalicarb (one mouse, one rat), and concluded that iprovalicarb was "likely to be carcinogenic to humans" (for the discussion of iprovalicarb throughout these comments, refer to EPA CARC Iprovalicarb 2002).

In a 24-month Wistar rat study, high-dose animals were fed 1109.6/1379.7 mg/kg bw/day. In male rats, there was a statistically significant increase in osteosarcomas in the high-dose group (3/60), with none in the control or other treatment groups. High-dose findings drove a statistically significant trend as well. The only statistically significant finding in female rats was a trend of increasing thyroid gland follicular cell tumors (adenomas and carcinomas combined) that was also driven mainly by high-dose findings: 0/49, 0/49, 2/48, 3/48 (control to high-dose). EPA did not discount findings at the high dose because it exceeded 1,000 mg/kg bw/day, as it did with glyphosate. Instead, EPA followed its guidelines by assessing biological effects. There were no treatment-related adverse impacts on survival in either males or females at the high dose. High-dose males experienced few and mild adverse effects, while a somewhat greater range and severity of adverse effects were noted in female high-dose and in some cases mid-dose rats. EPA concluded that the highest dose was adequate and not excessive for carcinogenicity testing.

In a 105-week study in $B6C3F_1$ mice, high-dose animals were fed 1566.8/2544.0 mg/kg bw/day (M/F) iprovalicarb. Although tumors were found in the liver, lung, pituitary gland, hematopoietic tissues and the Harderian gland, there was no evidence of treatment-related tumor increases in either sex at any of the dose levels. EPA did not declare the high doses in this study to be excessive merely because they exceeded 1,000 mg/kg bw/day, as it did with glyphosate. Instead, EPA assessed biological effects as per its guidelines. Signs of kidney and liver toxicity were observed mainly in high-dose groups of both sexes. EPA concluded that the dosing was adequate because of the observed signs of kidney and liver toxicity, but not excessive because the toxic symptoms were not severely adverse, and there were no treatment-related adverse effects on survival.

Iprovalicarb's hazard classification as "likely to be carcinogenic to humans" is based almost entirely on tumor findings in rats fed 1.1 (male) to 1.4 (female) times the "limit dose." Nowhere did EPA suggest that these tumor findings should be rejected, discounted, or given less weight

merely because they occurred at a dose that exceeded the arbitrary "need not exceed" level of 1,000 mg/kg bw/day. EPA likewise raised no objections to the high-dose mouse group receiving still higher multiples of the "limit dose" of iprovalicarb – namely, 1.6X / 2.5X (M/F). Although no treatment-related tumor findings were made in this mouse study, EPA's evaluation makes it clear that such findings in the high-dose groups would have been given full weight, had they occurred.

These were the only two carcinogenicity studies evaluated by EPA. Both were assessed for dosing, and both were found to employ adequately high, but not excessive, doses based on EPA guidelines. The EPA reviewers made no suggestion that the dosage levels for the high dose or other treatment groups should somehow correspond to anticipated levels of human exposure, nor that high-dose findings should be discounted because humans are (presumably) not exposed to such high levels of the pesticide, as the Agency did in its glyphosate evaluation.

2.0 Other Aspects of Animal Studies

2.1 Criteria for trend evaluation

In at least four of the glyphosate animal studies, EPA dismissed tumor findings primarily or partly due to absence of a monotonic dose-response pattern.[3] In so doing, EPA discounted statistically significant trends of increasing tumors with increasing dose, in violation of its guidelines. Nowhere in its evaluations of isoxaflutole or iprovalicarb does EPA demand that tumor findings fit a perfect monotonic dose-response pattern. Instead, EPA follows its guidelines by using statistical procedures (e.g. Cochran-Armitage trend test, Fischer exact test) to determine the significance of tumor trends and tumor incidence differences between treatment groups and controls.

2.2 Findings regarded as sufficient for classification as "likely carcinogenic"

Properly interpreted, at least 4 of 7 rat studies and 5 of 5 mouse studies provide evidence of glyphosate's carcinogenicity.[4] This constitutes more than sufficient evidence for a hazard classification of glyphosate as "likely to be carcinogenic to humans." In fact, just one positive

[3] See Center for Food Safety's prior comments, referenced above, on Lankas (1981), Stout & Ruecker (1990), Brammer (2001) and Atkinson et al. (1993b). The term is used here in its literal sense. For instance, in Lankas (1981), EPA dismissed a statistically significant trend in testicular tumors in male rats of 0/50, 3/47, 1/49, 6/44 (control, low, mid-, high-dose) in large part because because the low-dose incidence exceeded that of the mid-dose group, rather than fitting a perfect monotonic pattern of stepwise increase from control to high-dose.

[4] See Center for Food Safety's prior comments, referenced above, Sections 3-5. CFS's tally of positive and total studies differs from that given by EPA because we exclude inappropriate studies and find treatment-related effects in studies judged negative by EPA.

animal study can suffice for this classification according to EPA guidelines (see below, criteria 2 to 5).

From: EPA (2005): Guidelines for Carcinogen Risk Assessment, pp. 2-54 to 2-55:
Supporting data justifying the hazard classification "likely to be carcinogenic to humans"

1) An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments;
2) An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
3) A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset;
4) A rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
5) A positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

The available animal evidence regarding the carcinogenicity of isoxaflutole and iprovalicarb consisted of just two studies (mouse and rat) for each. Isoxaflutole's classification as likely carcinogenic was based on positive findings of liver tumors in both sexes of both species, and a second tumor site (thyroid) in male rats only. In contrast, iprovalicarb's classification of "likely carcinogenic" was based on positive findings in just one rat study, and despite a second study in mice with entirely negative findings. The primary evidence in the rat study was a low incidence of a rare tumor type (osteosarcoma) in high-dose males only; the only other statistically significant finding was a trend ($p = 0.0228$) of increasing thyroid follicular cell tumors (adenomas and carcinomas combined) in females: 0/49, 0/49, 2/48, 3/48 (control to high dose). These tumors are regarded as uncommon, but not rare, in female Wistar rats.[5]

In EPA's evaluation of glyphosate, the Agency has applied unreasonably stringent standards for what constitutes significant findings of carcinogenicity, both with respect to what constitutes a

[5] Two rare tumor types in female treatment groups provided additional evidence, though neither occurred at incidences that were increased in a statistically significant manner versus controls.

significant trend (monotonic dose-response[6] vs. Cochran-Armitage trend test) and overall evidence demanded for a hazard classification as "likely to be carcinogenic to humans." This is illustrated by the very different standards that EPA applied in its evaluations of two other pesticides: isoxaflutole and iprovalicarb. Comparison of the three carcinogenicity assessments reveals clearly that the the latter two are guideline-compliant, while that of glyphosate violates the Agency's carcinogen assessment guidelines in several important respects.

3.0 Mutagenicity and Other Mechanistic Evidence

3.1 Isoxaflutole

Isoxaflutole was classified by EPA as likely to be carcinogenic to humans despite testing negative for mutagenicity in all four assays reported by the Agency:

1) In vivo mouse micronucleus assay
2) *Salmonella typhimurium* reverse mutation assay (Ames test) involving four *S. typhimurium* strains, with or without S9 activation
3) TK+/- mouse forward gene mutation assays using lymphoma cells in vitro, with or without S9 activation
4) In vitro cytogenetic assay for clastogenic activity in human lymphocytes, with or without S9 activation.

This is proper practice in light of the primacy of animal and human over mechanistic evidence, given our still very incomplete understanding of mechanisms of carcinogenicity.

3.2 Iprovalicarb

Iprovalicarb was classified as likely to be carcinogenic to humans despite testing negative for mutagenicity in all six assays reported by the Agency:

1) *Salmonella typhimurium* reverse mutation assay, four strains, with and without S9 activation
2) *Salmonella typhimurium* reverse mutation assay, five strains, with and without S9 activation, conducted with p-methyl-phenethylamine, a metabolite of iprovalicarb in rats
3) *In vitro* forward mutation assay in Chinese hamster lung fibroblasts, with or without S9 activation

[6] It should be noted that in at least two cases (Wood et al. 2009b & Sugimoto 1997), EPA denied the significance of statistically significant tumor incidence trends even though they also fit a monotonic dose-response pattern.

4) Chromosomal aberration assay in Chines hamster ovary cells, with or without S9 activation
5) *In vivo* cytogenetics (mouse bone marrow micronucleus assay)
6) *In vitro* unscheduled DNA synthesis in rat primary hepatocytes

This is proper practice in light of the primacy of animal and human over mechanistic evidence, given our still very incomplete understanding of mechanisms of carcinogenicity.

3.3 Comparison to glyphosate

The absence of mutagenic results in assays on isoxaflutole and iprovalicarb contrasts with limited evidence for glyphosate's mutagenic potential found by EPA and more extensive mechanistic evidence of carcinogenicity found by IARC for glyphosate and glyphosate formulations (evidence that included oxidative stress as well as mutagenicity) (Guyton et al. 2015; IARC 2015). Thus, EPA has more supportive mechanistic evidence of carcinogenicity for glyphosate than it does for two other pesticides it has found to be likely human carcinogens. The inability to determine a carcinogenic mode of action for isoxaflutole and iprovalicarb led EPA to apply a linear, low-dose extrapolation approach to determine the carcinogenic potency of these pesticides (for isoxaflutole, with respect to liver tumors, not thyroid tumors), an approach that would seem to be justified for glyphosate as well.

REFERENCES

EPA CARC Iprovalicarb (2002). Iprovalicarb – Report of the Cancer Assessment Review Committee. Environmental Protection Agency, April 11, 2002.

EPA CPRC Isoxaflutole (1997). Carcinogenicity Peer Review of Isoxaflutole. Carcinogenicity Peer Review Committee, Environmental Protection Agency, August 6, 1997.

EPA (2005). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency, EPA/630/P-03/001F, March 2005.

EPA (1998a). Health Effects Test Guidelines: OPPTS 870.4200 Carcinogenicity. Office of Prevention, Pesticides and Toxic Substances, EPA, 1998. Accessible from: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines. [EPA refers to these guidelines as OCSPP 870.4200. However, a google search on "OCSPP 870.4200" led directly to the website noted above, where the relevant link is to the guidelines referenced above with a different acronym: OPPTS.]

EPA (1998b). Health Effects Test Guidelines: OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity. Office of Prevention, Pesticides and Toxic Substances, EPA, 1998. Accessible from: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines. [EPA refers to these guidelines as OCSPP 870.4300. However, a google search on "OCSPP 870.4300" led directly to the website noted above, where the relevant link is to the guidelines referenced above with a different acronym: OPPTS.]

Guyton KZ et al. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncology 16: 490-491.

IARC (2015). Glyphosate Monograph, Volume 112, International Agency for Research On Cancer, World Health Organization. http://monographs.iarc.fr/ENG/Monographs/vol112/mono11.