

Exhibit 21

Genotoxic Potential of Glyphosate Formulations: Mode-of-Action Investigations

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A broad array of *in vitro* and *in vivo* assays has consistently demonstrated that glyphosate and glyphosate-containing herbicide formulations (GCHF) are not genotoxic. Occasionally, however, related and contradictory data are reported, including findings of mouse liver and kidney DNA adducts and damage following intraperitoneal (ip) injection. Mode-of-action investigations were therefore undertaken to determine the significance of these contradictory data while concurrently comparing results from ip and oral exposures. Exposure by ip injection indeed produced marked hepatic and renal toxicity, but oral administration did not. The results suggest that ip injection of GCHF may induce secondary effects mediated by local toxicity rather than genotoxicity. Furthermore, these results continue to support the conclusion that glyphosate and GCHF are not genotoxic under exposure conditions that are relevant to animals and humans.

KEYWORDS: Glyphosate; genotoxicity; mode of action

INTRODUCTION

The potential genotoxicity of glyphosate has been tested in a wide variety of *in vitro* and *in vivo* assays. No genotoxicity was observed in standard assays conducted according to international guidelines and Good Laboratory Practice (GLP) Standards. These assays are described briefly in Williams et al. (1), and the results have led to the conclusion that glyphosate does not pose a risk for the production of heritable or somatic mutations in humans (1–6). The original Roundup formulation and subsequent glyphosate-containing herbicide formulations (GCHF) have also been evaluated for genotoxic responses in several assays. Although a number of studies conducted according to international guidelines and GLP Standards show that these materials are not genotoxic (1), a few other studies have reported positive effects.

Apparent evidence of DNA adducts in the liver and kidneys of CD-1 mice was reported (7) when a formulation that was identified as “Roundup” (30.4% glyphosate, purchased from Monsanto, Italy) was administered intraperitoneally (600 mg/kg) using dimethyl sulfoxide (DMSO)/olive oil as a vehicle.

However, no DNA adducts were observed following intraperitoneal (ip) injection of isopropylamine salts of glyphosate. In contrast, ip injection of CD-1 mice with analytical grade glyphosate or the same “Roundup” formulation resulted in an increased incidence of alkali-labile sites in DNA from liver and kidney (8). The effects reported in the latter study (8) were observed at 300 mg/kg with glyphosate and at 900 mg/kg for GCHF, including a dramatic increase in the number of 8-hydroxydeoxyguanine (8-OHdG) residues in DNA from liver cells after treatment with glyphosate but not the GCHF; opposite results were found in the kidney. All of these changes were observed only under unrealistic exposure conditions (very high dose levels administered by an irrelevant route of exposure for an agricultural herbicide).

To better understand the significance of these results (7, 8), four separate but inter-related assays were undertaken to determine if high-dose ip administration produces toxicity that may be responsible for the observed changes via secondary effects, rather than direct genotoxicity, and whether a more relevant (oral) route of exposure produces the same toxic responses as those seen with ip administration. The first assay was performed to understand the relevance of findings reported by Bolognesi et al. (8) by investigating the degree of liver and kidney toxicity that occurred under the dosing conditions used by those investigators. Similarly, another assay was conducted to understand the relevance of findings reported by Peluso et al. (7); this assay also examined whether the vehicles used in their studies (DMSO/olive oil) contributed to the hepatic

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July 25, 2002

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U. S. Environmental Protection Agency
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1921 Jefferson Davis Highway
Arlington, VA 22202-4501

Attention: Mr. James A. Tompkins
Team Leader (25)

**Subject: Toxicity Data for a Glyphosate Formulation Dosed IP to Mice
RD 1587: Supplemental Toxicology Studies**

Dear Mr. Tompkins:

MON 35050 is a 41% by weight isopropylamine glyphosate formulation that was historically sold under the Roundup brand in specific European countries, among them, Italy. MON 35050's surfactant component was anionic in nature and therefore different from that used in other Monsanto glyphosate products sold in most countries, including the US.

In 1997 and 1998, two related Italian research groups authored papers in the open literature reporting that they had measured genotoxic effects of Italian Roundup. Their work had been conducted via intraperitoneal (IP) injection of large quantities of the Roundup formulation in an olive oil/DMSO carrier, and had focused on detection of clastogenicity and DNA adduct formation in treated liver and kidney tissues. Citations for these articles can be found in the list of references in the submitted volumes.

These articles caused questions from toxicologists and have subsequently served as one basis for arguments against Monsanto's Roundup Ready crops when advocacy groups opposing biotechnology lobby regulators or attempt to enhance public concern.

Monsanto has never accepted these Italian studies as authentic and reliable evidence of genotoxic activity for MON 35050 based on a variety of technical reasons, combined with our knowledge that many other studies using established testing methods, including chronic



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dosing, had convincingly shown a lack of genotoxic and carcinogenic activity for glyphosate. In addition, no genotoxicity had been observed in a number of other studies with a variety of other types of surfactants and formulations. However, the presence of a different surfactant in the Italian commercial formulation never permitted a completely unambiguous conclusion.

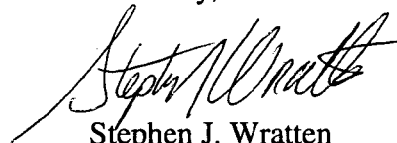
Beginning in 1999, for stewardship purposes, Monsanto undertook to repeat aspects of these studies, and investigate other explanations for the reported outcomes. The enclosed two reports and the attached summary describe the results of those investigations. The attached summary describes additional follow-up experimental work that has not been described in a final report format. Monsanto wishes to share this information with the Agency.

This evidence together demonstrates that any genotoxic responses that may have occurred in the literature studies were secondary to widespread and marked cytotoxicity in the animals and were not indicative of primary genotoxic activity. The IP dosing route and the influence of the carrier (olive oil / DMSO) produced extreme kidney and liver toxicity / cytotoxicity. A variety of clinical, biochemical, and physiological parameters were measured to document these toxic effects. Such toxic effects were reduced or eliminated when a water carrier was used or when dosing was administered by oral exposure. 8-hydroxydeoxyguanosine (indicative of DNA adducts) was not detectable in the Monsanto experiments. The reports conclude that the experimental conditions used by the Italian researchers are not appropriate to assess genotoxicity, due to the secondary artifacts resulting from generalized organ cytotoxicity. The follow-up experiment, described in the attached summary, established that the organ toxicity effects were caused by the MON 35050 formulation matrix alone (without glyphosate), supporting a further conclusion that these effects are a non-specific response attributable to the dosing method, vehicle, perhaps in combination with the formulation's surfactant ingredient, but are not in any way linked to glyphosate itself. Overall, this body of work continues to reinforce that glyphosate and its formulations are not genotoxic in assays that are relevant to human and animal exposures.

The findings from this Monsanto work are also summarized in *The Toxicologist* (Volume 60, Number 1, March 2002, Abstract 262).

If you have any questions on this matter please feel free to contact me through Dr. Marsha C. Gray (202-783-2878) or by direct phone (314-694-1582), fax (314-694-4028), or electronic mail at stephen.j.wratten@monsanto.com.

Sincerely,



Stephen J. Wratten
Manager, Registrations

cc: M. C. Gray

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