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CALUX AND HIGH RESOLUTION GC/MS ANALYSIS OF DIOXIN-LIKE COMPOUNDS IN CHLOROPHENOXY PESTICIDE FORMULATIONS

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Introduction

An epidemiology study to evaluate the health effects of pesticide exposure is being conducted by the University of Minnesota on a cohort of pesticide applicators from the Red River Valley of Minnesota. An initial report from a sub-cohort of this study has suggested a correlation between pesticide exposure, testosterone levels, and the sex ratio of the applicators' offspring.[1] Because polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) have been known to effect the sex ratio of offspring[2,3] and are found in various chlorinated pesticides,[4,5,6] a number of pesticides commonly used in the Red River Valley of Minnesota were collected and assayed for dioxin-like activity by a reporter gene bioassay (CALUX). Of the twelve pesticides assayed, ten had measurable dioxin toxic equivalency (TEQ) and four had TEQs of over 1 ppb.[1] In order to confirm the presence of PCDD/Fs and provide a congener profile of dioxins in these pesticides, we have now performed high-resolution GC/high-resolution MS (HRGC-MS)analysis on several of these same pesticides. This paper will compare the results from the CALUX bioassay to that of conventional HRGC-MS analyses.

Materials and Methods

Pesticides. Samples of chlorophenoxy pesticide mixtures that were sprayed in the Red River Valley were collected in the years 1993 and 1998 under the guidance of the University of Minnesota. Samples were stored at 4°C until sent for analysis. Sub-samples of eight of these pesticides were shipped to Xenobiotic Detection Sytems and to the USDA, ARS, Biosciences Research Laboratory for analysis by the CALUX bioassay for dioxin-like TEQ and for HRGC-MS congener-specific dioxin analysis.

CALUX bioassay. The CALUX bioassay is a reporter gene assay in which a mouse hepatoma cell has been stably transfected with a vector that contains the luciferase gene under transactivational control of the Ah receptor.[7,8,9] Combined with a patent-pending sample processing system to reduce contamination with non-dioxin-like agonists for the Ah receptor, the CALUX bioassay can be used for estimation of TEQ contamination with dioxin-like chemicals of environmental, biological, and chemical samples.[10,11,12] In this study, a 0.5 g sub-sample of each herbicide mixture was dissolved in hexane and processed through our patent-pending cleanup procedure.[10] The isolated extract was exchanged into dimethyl sulfoxide and applied to 96 well plate monolayers of our genetically engineered cells and incubated for maximal induction of the firefly luciferase within the cells. Light produced by the firefly luciferase enzyme was measured with an assay kit from Promega and quantified on a Berthold Orion Microplate Luminometer. Dilution analysis was performed for range finding of the approximate concentration of the processed

sample that induced maximal luciferase activity for quantification in the CALUX bioassay. Dioxin-like TEQ activity was estimated from a standard curve of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using a four-parameter Hill equation and is reported as the mean ± the standard deviation of triplicate analyses.

HRGC-MS analysis. Prior to HRGC-MS analysis, 0.1 g of each sample was spiked with fifteen ^{13}C-labeled PCDD/Fs and three ^{13}C-labeled PCBs (recovery standards) and purified according to EPA Method 1613 using an FMS automated cleanup system (Fluid Management Systems, Waltham, MA). Herbicide samples that were hexane soluble were directly applied to the FMS system. Samples that were not readily soluble in hexane were partitioned between hexane and 2.0 M potassium hydroxide with rigorous shaking. The hexane layer was dried with sodium sulfate before being loaded onto the FMS system. A solvent blank was run with each set of samples. The PCDD/Fs and three co-planar PCBs were quantitated by EPA Method 1613. All values were blank-subtracted. WHO-TEFs were used to calculate TEQs.

Results and Discussion

Table 1 shows the TEQs obtained from the CALUX bioassay and from the HRGC-MS analysis of eight pesticide samples. For six of the samples, the TEQs from both methods correlated quite well, although the CALUX TEQs tended to be higher than the HRGC-MS results by 1.3–3 fold. Two pesticide samples (Bronate and Assure II) differed greatly in the response of the CALUX bioassay and the HRGC-MS. This divergence is most likely due to the inherent selectivities of the methods. While the HRGC-MS method is specific for only the tetra- through octa-chlorinated dioxins and furans, the CALUX bioassay has a broader specificity based on induction of a firefly luciferase gene in response to binding and activation at the aryl hydrocarbon receptor. As a consequence, the CALUX bioassay can respond to numerous brominated dioxin-like compounds and some non-dioxin compounds that activate the aryl hydrocarbon receptor pathway. The active ingredients in Bronate and Assure II contain halogenated aromatic moieties that may possess some affinity for the aryl hydrocarbon receptor and elicit a CALUX response. However, these ingredients appear unlikely to form PCDD/Fs as by-products during processing.

In general, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD dominated the TEQs of the pesticides. No significant amounts of co-planar PCBs were detected in any samples. In addition to the 2,3,7,8-substituted dioxins and furans, other isomers were also present in the formulations. The homolog distribution profile for 2,4-D LV4 is shown in Figure 1. A similar profile was seen for each of the other pesticides. The polychlorinated furans dominated the pattern. Although TCDF was the major homolog group, no 2,3,7,8-TCDF was found in any of the pesticides. Among the 2,4,-dichlorophenoxyacetic acid (2,4-D) type of formulations, TEQs ranged from near background to 2,600 ppt. Schecter et al. have reported TEQs ranging from 1-850 ppt for 2,4-D formulations from various countries.[5] The large variability seen from sample to sample may arise from differing degrees of purity in the raw starting materials. A similar explanation has been proposed by Masunage et al. who found elevated TEQs (up to 900 ppb) in chloronitrofen (CNP).[6] CNP is produced from 2,4,6-trichlorophenol, but impurities in the chlorophenol can give rises to toxic dioxin and furan byproducts.

In conclusion, the CALUX bioassay appeared to be a good screening tool for dioxin-like activity in a variety of pesticides. Compared to HRGC-MS, the CALUX TEQs were generally higher. Given the nature of the CALUX assay, however, it may provide a more accurate assessment of the

potential biological activity of pesticide formulations than the highly selective HRGC-MS method. The results of this study also confirm the presence of toxic PCDD/Fs in certain 2,4-D pesticides and support the need for more investigation into possible human health effects.

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Pesticide	Collection Year	Active Ingredients	CALUX TEQ (n=3)	HRGC-MS TEQ
2,4-D Amine	1993	2,4-D dimethylamine, 46.9%	26.2 ± 0.5	8.7
See 2,4-D	1993	2,4-D isooctylester, 61.7%	1637 ± 212	731
2,4-D LV4	1993	2,4-D isooctylester, 66.2%	3392 ± 257	2627
2.4-D LV6	1993	2,4-D isooctylester, 88.8%	1882 ± 311	1379
Weedone LV4	1998	2,4-D isooctylester, 67.2%	45.7 ± 4.1	27.7
Tiller	1998	MCPA-2EH ester, 32.1% 2,4-D isooctylester, 10.4% Fenoxaprop-p-ethyl, 4.4%	34.2 ± 4.1	19.8
Bronate	1998	Bromoxynil octanoic acid ester, 31.7% MCPA isooctyl ester, 34%	800 ± 180	0.3
Assure II	1998	Quizalofop-p-ethyl, 10.3%	1668 ± 419	4.1

Table 1. Composition and TEQs (ppt) of the chlorophenoxy pesticides analyzed in this study. 2,4-D = 2,4-dichlorophenoxyacetic acid; MCPA = 4-chloro-2-methylphenoxyacetic acid; EH = ethylhexyl.

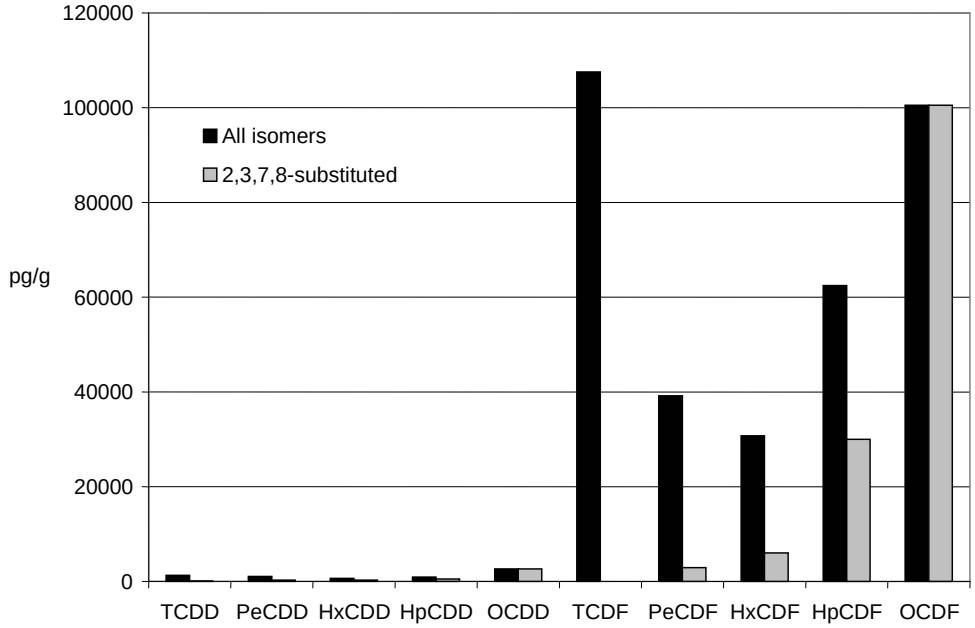

Figure 1. Homolog profile of 2,4-D LV4.

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