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Absence of Transformation by Diflubenzuron in a Host-Mediated Transplacental Carcinogen Assay[1]

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The search for insecticides with specific or selective activity rather than broad spectrum activity has led to increased interest in new compounds, including the chitin-synthesis inhibitors. One of these chemicals, diflubenzuron, has been shown to be effective against the gypsy moth and may also be useful against a variety of other insects including certain beetles, weevils, flies and rust mites.

Diflubenzuron is a relatively stable compound on foliage, depending upon the particle size of the residue deposit. Although its degradation in the environment was cited by MARX (1977), its possible persistence in soil and water suggest the need for conducting a variety of tests for biological effects of the chemical and its metabolites. The compound has been considered to show only minimal toxicity for fish, birds and mammals but several reports have raised questions about potential risks. SEUFERER et al. (1979) reported that diflubenzuron could be degraded by eucaryotic microorganisms and that three metabolites were borderline mutagens in the *Salmonella* mutagenicity test. NIMMO et al. (1979) found diflubenzuron to be acutely and chronically toxic to the estuarine crustacean, *Mysidopsis bahia*. Other workers (MIURA & TAKAHASHI 1974, MIURA & TAKAHASHI 1975, CUNNINGHAM 1976) reported the chemical killed mayflies, cladocerans, clam, shrimp, and tadpole shrimp, and reduced reproduction in brine shrimp at concentrations of 0.01 to 2.0 µg/L. Because the chemical or its metabolites may persist for some time in the environment, we tested diflubenzuron in a short term host mediated transformation assay to obtain information on its possible carcinogenicity.

METHODS AND MATERIALS

A transplacental transformation assay, originally described by DI PAOLO et al. (1973) and utilized by QUARLES et al. (1979 a,b) to test several pesticides and nitrosated derivatives was used to investigate possible transformation of mammalian cells in culture by diflubenzuron. Timed pregnant hamsters were obtained from Engle Lab Animals (Farmersburg, IN) or Charles Rivers

[1]Mention of pesticide does not constitute a recommendation for use by USDA nor does it imply registration under FIFRA as amended.

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Laboratory (Boston, MA). Animals were injected intraperitoneally with test chemicals, solvents, or saline on the tenth day of gestation. Concentrations of chemicals were as shown in Table 1. Known carcinogens benzo(a)pyrene, diethylnitrosamine and dimethylnitrosamine were used as positive control chemicals. Solvents were tested alone to insure lack of solvent-induced response. Three days after injection (13th day of gestation), animals were sacrificed and fetal cell cultures prepared by previously published techniques (DI PAOLO et al. 1973, QUARLES et al. 1979 a,b). Cell cultures were prepared and maintained in Dulbeccos Minimal Essential Medium supplemented with fetal bovine serum (Sterile Systems Inc., Ogden,UT) and antibiotics (penicillin and streptomycin). Cells were subcultured every 3-5 days and plated periodically at low density for detection of transformed morphology, and suspended in 0.3% agar for detection of growth of colonies. The percent transformation was determined by counting the number of transformed colonies and the total number of colonies on the dish after fixation of the cells with methanol and staining with Giemsa stain. Positive growth in agar was determined by examining the agar plates and measuring colonies. Colonies of diameter $\geq$ 0.1 mm were scored as positive. Colony forming ability was determined as the number of positive colonies /number of cells plated, expressed as a percent. Routinely, cultures were tested at subcultures 3 and 5 or 6 for both morphological transformation and ability to grow in agar.

RESULTS

A summary of results obtained by the host-mediated transplacental carcinogen assay is shown in Table 1. There was no evidence of transformation of hamster fetal cells induced by diflubenzuron, even when very high concentrations of the chemical (50 mg/100 g body weight) were injected. A yolk extract of eggs from chickens which had received 250 ppm diflubenzuron for life was also negative for cellular transformation. The assay did detect transforming activity in the three positive control chemicals. In no case did the solvent (dimethylsulfoxide) or the saline control induce transforming activity. Likewise, the untreated control tests indicated no spontaneous transformation. These results are summarized in Table 2.

DISCUSSION

Diflubenzuron was found to induce neither morphological transformation nor the ability to grow in 0.3% agar when used to treat hamster fetal cells in a transplacental assay. These two tests have been shown to have a high degree of correlation with tumorigenicity of mammalian cells upon injection into an appropriate host, and the negative response observed provides strong suggestive evidence that diflubenzuron is not likely a moderate or strong carcinogen. Although failure to transform cells at a dose as great as 50 mg/100 g body weight is not proof of noncarcinogenicity, when compared to the positive reaction induced in

TABLE 1. Effect of Diflubenzuron on Hamster Fetal Cells in the Host Mediated Transplacental Assay.

Treatment	Concentration mg/100 g body weight	Transformed colonies	Total Number of colonies	Transformation rate (%)[a]	Growth in Agar[b] Result	CFE(%)
Diflubenzuron	1.0	0	1494	0	Negative	0
Diflubenzuron	20.0	0	1428	0	Negative	0
Diflubenzuron	20.0	0	1456	0	Negative	0
Diflubenzuron	50.0	0	1364	0	Negative	0
Diflubenzuron, Yolk Extract[c]	0.1 mL	0	1518	0	Negative	0

[a] Transformation rate defined as (number transformed colonies/total number of colonies) x 100.

[b] One thousand to fifty thousand cells plated in 0.3% agar in 60 mm tissue culture dishes. Scored for growth of 3-dimensional colonies, diameter $\geq$ 0.1 mm. Ten to 20 dishes examined. CFE = colony forming efficiency: number of colonies positive for growth/number of cells plated times 100.

[c] Injected 0.5 mL of yolk extract from chickens fed 250 ppm diflubenzuron for life. Yolk contained 97 ppm diflubenzuron.

TABLE 2. Effect of Chemicals of Known Activity in the Host Mediated Transplacental Assay

Treatment	Concentration mg/100 g body weight	Transformed colonies	Total Number of colonies	Transformation Rate (%)[a]	Growth in Agar[b] Result	CFE (%)
Benzo(a)pyrene	3.0	12	1251	1.0	Positive	0.7
Benzo(a)pyrene	3.0	15	1646	0.9	Positive	1.1
Dimethylnitrosamine	2.0	16	1471	1.1	Positive	1.5
Diethylnitrosamine	2.0	19	1441	1.3	Positive	2.1
Dimethylsulfoxide	0.2 mL	0	1218	0	Negative	0
Dimethylsulfoxide	0.4 mL	0	1358	0	Negative	0
Saline	0.2 mL	0	1585	0	Negative	0
Saline	0.4 mL	0	1440	0	Negative	0
Untreated	NA[c]	0	1537	0	Negative	0
Untreated	NA	0	1212	0	Negative	0

[a] Transformation rate defined as (number transformed colonies/total number of colonies) x 100.

[b] One thousand to fifty thousand cells plated in 0.3% agar in 60 mm tissue culture dishes, scored for growth of 3-dimensional colonies, diameter $\geq$ 0.1 mm. Ten to 20 dishes examined. CFE = colony forming efficiency: number of colonies positive for growth/number of cells plated times 100.

[c] NA = not applicable.

this assay system by known carcinogens such as benzo(a)pyrene, nitrosamines, dimethylbenzanthracene, nitroquinoline-1-oxide, urethane, and β-propiolactone at concentrations ranging from 0.2 to 3 mg/100 g body weight (QUARLES et al. 1979b) the non-reactivity of diflubenzuron is impressive. Similar negative results obtained from a realistic biological sample (yolk extract) containing 97 ppm diflubenzuron are also impressive. Although all the metabolites of diflubenzuron are not fully characterized as to carcinogenicity, it should be noted that the transplacental host mediated assay would be expected to detect activity of chemicals requiring metabolic activation as well as those which are direct acting. These results do not prove non-carcinogenicity for humans but they do indicate diflubenzuron may be negative or relatively low in oncogenic potential.

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