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THE EFFECT OF THE PESTICIDES, DEXON, CAPTAN AND ROUNDUP, ON SISTER-CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES IN VITRO

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Summary

3 pesticides at varying concentration were tested for the induction of SCE in human lymphocytes in vitro. The fungicide, Dexon, sodium (4-(dimethylamino)phenyldiazene sulfate, was found to cause the greatest increase in SCE frequency and the response is dose related. The herbicide, Roundup, isopropylamine salt of *N*-(phosphonomethyl)glycine, had the least effect on SCE requiring the use of much higher concentrations to produce an effect. Limited results were obtained with the fungicide Captan, *cis*-*N*-((trichloromethyl)thio)-4-cyclohexene-1, 2-dicarboximide because of toxic levels of either the fungicide or solvent used.

The fungicide, Captan, *cis*-*N*-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarboximide, is commonly used in agriculture for control of fungal diseases and for prevention of spoilage of agricultural products in transit. It is noteworthy that Captan is allowed to remain on harvested crops in the U.S. at levels of 100 ppm, the highest of any organic pesticide [19]. The fungicide Dexon (also called Lesan or Fenaminosulf), sodium (4-(dimethylamino)phenyl)diazene sulfonate, is recommended for use on germinating seeds and seedlings of a variety of crops. The mutagenicity and carcinogenicity of these substances is therefore of concern. Dexon has been shown to be mutagenic in prokaryotic and eukaryotic test systems [9,14,18,20,24]. Captan has been shown to produce point mutations in bacteria [6,9,14,19] and in Chinese hamster cells [2] as well as to increase the frequency of chromosomal aberrations [13]. Other data on the mutagenicity of Captan shows conflicting results in mammalian systems [4] and in Drosophila [11,18]. The herbicide Roundup, isopropyl-

amine salt of *N*-(phosphonomethyl)glycine, is relatively new on the market and, although no published data on its mutagenicity/carcinogenicity have been reported, it is considered to be relatively innocuous.

Induction of SCE in mammalian cells is a sensitive method of measuring the potential mutagenicity of chemicals [1,3,5,7,10,12,15,21–23]. SCEs have been found to increase with increasing concentrations of mutagens and carcinogens without necessarily increasing chromosome aberrations [17]. There is also a correlation between the induction of SCE and the induction of single-locus mutations in mammalian cells [5].

In this study the SCE index was used as a measure of the potential mutagenicity/carcinogenicity of these pesticides by applying varying concentrations to human lymphocytes and determining subsequent SCE frequencies. Ethylmethane sulfonate (EMS) was used as a positive control since it has been demonstrated to be mutagenic and to cause a significant increase in SCE frequency [5,7,15]. Results are imcomplete for Captan as a result of cellular toxicity experienced at the higher levels of concentration.

Materials and methods

Human lymphocytes were cultured in McCoys 5A medium (Gibco) with the addition of 10% fetal calf serum, 1% PHA (Gibco), 1% pen-strep solution (10 000 units pencillin and 10 000 μg streptomycin/ml), and 30.7 μg/ml 5-bromodeoxyuridine (10^{-4} M). Also, excepting controls, each 5-ml culture contained in addition either EMS, Dexon or Captan in concentrations ranging from 10^{-5} to 10^{-3} M or Roundup at 65× higher concentrations (Table 1). Captan was dissolved in 7% ethanol followed by a 1 : 4 dilution in water. All other chemicals were dissolved in distilled water.

TABLE 1

SISTER-CHROMATID EXCHANGE FREQUENCIES IN HUMAN LYMPHOCYTES EXPOSED TO VARYING CONCENTRATIONS OF PESTICIDES

Treatment	Concentration mg/ml medium (M)	Subject No. 1		Subject No. 2	
		Mean SCE	Sign.	Mean SCE	Sign.
Control		14.18 ± 2.9		17.06 ± 3.5	
EMS	1.24 (10^{-5})	14.68 ± 3.5	0.5	16.50 ± 2.8	0.30
EMS	12.4 (10^{-4})	16.86 ± 3.4	0.001	18.22 ± 3.1	0.100
EMS	124.0 (10^{-3})	21.84 ± 4.0	0.001	24.68 ± 4.7	0.001
Dexon	2.5 (10^{-5})	18.52 ± 3.4	0.001	17.75 ± 3.2	0.50
Dexon	25.0 (10^{-4})	19.84 ± 3.9	0.001	19.22 ± 3.4	0.01
Dexon	250.0 (10^{-3})	26.50 ± 4.1	0.001	26.42 ± 4.1	0.001
Captan	3.0 (10^{-5})	18.90 ± 3.5	0.001	18.44 ± 3.4	0.05
Captan	30.0 (10^{-4})	n.g. [a]		n.g. [a]	
Captan	300.0 (10^{-3})	n.g. [a]		n.g. [a]	
Roundup	0.25 (65×10^{-5})	16.48 ± 3.7	0.001	19.50 ± 2.9	0.001
Roundup	2.5 (65×10^{-4})	18.90 ± 3.3	0.001	18.14 ± 3.2	0.10
Roundup	25.0 (65×10^{-3})	n.g. [a]		n.g. [a]	

[a] No growth.

2 subjects were chosen to act as regular donors of blood cells. 5 ml culture tubes were inoculated with 0.2 ml of a gravity settled leukocyte suspension and incubated for 72 h. Harvesting was done by traditional methods and cells were fixed on slides with methanol : acetic acid (3 : 1). After overnight drying slides were stained by the FPG technique [16]. For each sample and concentration, 50 well spread and completely differentially stained metaphases were analyzed for SCE frequency from each subject.

Results

Results for the 3 pesticides tested and the controls are shown in Table 1 with the mean SCE frequency of 2 experiments, standard devidations and significance levels as determined by Student's *t*-test. In addition, dose—response curves for EMS and Dexon are presented in Fig. 1. Similar data for Captan and Roundup are not presented because the higher concentrations are toxic (Table 1).

Paired Student's *t*-test were determined for all pairs of data in both subjects. EMS at the lowest concentration did not significantly increase SCE over the controls. The highest concentrations of EMS (10^{-4} and 10^{-3} M) did significantly increase the level of SCE over the controls and over the 10^{-5} M concentrations in both subjects (Table 1 and Fig. 1).

In subject number one, Dexon increased SCE significantly at all concentrations tested. In subject number two, Dexon did not significantly increase the SCE at the lowest concentration but did produce a significant increase at the higher concentrations. At the 3 concentrations tested, Dexon produced a significantly higher level of SCE than EMS (P 0.05) at each of the concentrations tested in both subjects.

Captan at the lowest concentration (10^{-5} M) significantly increased SCE

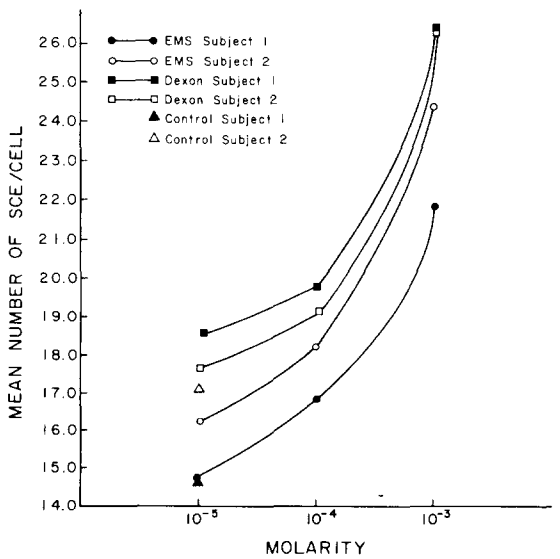

Fig. 1. SCE dose—response for varying concentrations of Dexon and EMS.

levels over controls and EMS at the same concentration (P 0.05).

Direct comparisons of the Roundup results to those of EMS and Dexon are not feasible because the concentrations used for Roundup were 65× higher. The results at the concentrations for which data is available are significantly higher than the controls except for the 65× 10^{-4} M concentration in the second subject. The slightly reduced effect of the higher concentration in the second subject is not easily explained unless there is a toxicity effect removing some affected cells from the population.

Discussion

Carrano and co-workers [5] recently reported a linear relationship between the induction of SCEs and the production of mutations. The increased SCE frequency found in human lymphocytes exposed to Dexon corresponds to previous data on the mutagenicity of Dexon [14,18—20,24] and lend further support to the use of the SCE index in mutagenicity testing. In our test, the SCE response is dose-dependent (Fig. 1) as reported for other mutagens [5,15]. The SCE induced in lymphocytes exposed to Dexon are significantly higher than those exposed to the same concentration of EMS, a well known mutagen. Dexon is a potent mutagen and inducer of SCE but it has recently been reported not to be carcinogenic in a test involving 50 treated rats and mice (NCI Report). Based on the previous mutagenicity tests and the mammalian SCE results reported here the carcinogenicity tests should probably be repeated.

Captan has been demonstrated to be mutagenic in several test systems [2,4,6,9,13,14,19,20] and based on this data we would expect this chemical to evoke a significant increase in the SCE index. Unfortunately this could only be tested at the lowest concentration because both higher concentrations inhibited growth of human lymphocytes. The limited data shows that the SCEs are significantly higher when cells are exposed to Captan than to EMS at the same concentration. The United States Environmental Protection Agency has recently triggered Captan for RPAR (Rebuttable Presumption Against Registration) because of its mutagenicity, possible oncogenicity (NCI Report) and teratogenicity. Although not necessarily a consequence of presumption, triggering may result in restricted use, suspension, or ultimate cancellation of registrations of the pesticide for agricultural use. The presumption and ensuing rebuttal and evaluation of risks versus benefits is called RPAR [8].

To the best of our knowledge Roundup has not previously been tested for its effect on DNA. The results reported here show significant increases in the SCE index upon exposure to Roundup, but only when concentrations were increased 65× over those used for the other pesticides. Although our conclusions are tentative because of the apparent toxicity, we suggest that this chemical is at most weakly mutagenic based on the SCE test. These results suggest that Roundup should be evaluated in other genetic tests that measure point mutations and chromosome aberrations. Furthermore, this herbicide is probably not a biological hazard except during application because it is inactivated on contact with the soil.

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