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Phenoxyacids as Inhibitors of Testicular DNA Synthesis in Male Mice

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Phenoxyacids are used in large amounts as weed killers in agriculture and forestry. In several papers their mutagenic properties have been investigated and positive (DAEVRING and HULTGREN 1977, MAJUMDAR and GOLIA 1974, VOGEL and CHANDLER 1974, ZETTERBERG et al. 1977) as well as negative (ANDERSEN et al. 1972, EPSTEIN et al. 1972, FAHRIG 1974, JENSSEN & RENBERG 1976) results have been reported. Extensive carcinogenicity experiments, however, have not been described. In view of the widespread application of phenoxyacids we included them in our survey of compounds by means of the testicular DNA synthesis inhibition test (FRIEDMAN and STAUB 1976). This test correlates well with the carcinogenicity of chemicals (SEILER 1977) and an unexpected positive result in this test thus would call for a thorough investigation of the carcinogenic potential of such a substance.

MATERIALS and METHODS

2,4-Dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and -isooctylester (2,4,5-T-ester), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2-methyl-4-chlorophenoxypropionic acid (MCPP), resp., were from registration samples collected at the Federal Research Station. 2,4-Dichlorophenoxypropionic acid (2,4-DP) was obtained from Serva (Heidelberg, FRG), and (^{3}H)- and (^{14}C)-thymidine from The Radiochemical Centre (Amersham, UK). Radioactivity of samples was measured in a toluene-triton X-100 (2:1) cocktail containing 0.4% BBOT (Ciba-Geigy, Basle, Switzerland) as fluor by means of a Beckman liquid scintillation counter. DNA content was estimated photometrically by the diphenylamine method (ASHWELL 1957). Inhibition of testicular DNA synthesis was measured as described (FRIEDMAN and STAUB 1976) with a slight modification: One hr before dosing the male mice orally with the test substance they received 1 µCi (methyl-^{14}C)-thymidine i.p. Three (to 96 - according to special situations) hr after the application of the test compounds they were given 10 µCi (methyl-^{3}H)-thymidine i.p. and 0.5 hr later they were killed, the testes removed, homogenized in 5% trichloroacetic acid (TCA), washed successively with 5% TCA twice, methanol, methanol-chloroform (1:1), methanol, 5% TCA and finally hydrolyzed 30 min in 5% TCA at

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95°. After cooling and centrifugation aliquots of the supernatant were taken for the determination of radioactivity and DNA content. The ratios of tritium counts per µg DNA and of tritium counts per ^{14}C-count were then compared with the resp. values from the concurrent controls.

RESULTS and DISCUSSION

Of the five phenoxyacids tested four yielded positive results, i.e. they depressed thymidine uptake significantly. Only 2,4-DP was not significantly positive (see Table 1). As there exists a high correlation between inhibition of testicular DNA synthesis and carcinogenicity by a chemical agent, the question arises immediately, whether phenoxyacids should be suspected of carcinogenic activity. One pitfall, however, lies in such a conclusion. It is known that inhibitors of enzymatic reactions in the DNA synthetic pathway do also depress thymidine incorporation, and it might be possible also for other cytotoxic substances to produce the same effect, although neither KCN nor 2,4-dinitrophenol act as inhibitors of DNA synthesis (FRIEDMAN and STAUB 1976). There is, however, a way to avoid these difficulties, which is based on the following assumptions. In order to exert their biological effects, carcinogens and mutagens have to bind to DNA. These alterations of the DNA structure then lead to a decreased template activity and to an inhibition of DNA synthesis. This effect will persist even after complete excretion of the unbound part of this compound and DNA synthesis will only be restored to control levels by repair of the damaged sites in the DNA. On the other hand enzyme inhibition is dependent on a certain concentration of the substance in the cell. Upon excretion of the inhibitor enzymes will resume

TABLE 1

Inhibition of testicular DNA synthesis by various phenoxy acids

Compound	Concentration (mg/kg)	Inhibition (%)	p <
2,4-D	200	29	0.05
2,4-DP	200	14	-
2,4,5-T-acid	200	39	0.05
2,4,5-T-isooctylester	400	44	0.01
	200	31	0.05
	100	10	-
	50	1	-
MCPA	200	54	0.01
MCPP	200	60	0.01

their resp. functions according to the level of inhibitor remaining. That these assumptions are valid is shown in Figure 1. Whereas the alkylating activity of methyl methane sulfonate (MMS) in serum falls rapidly with a half life of about 30 min, the inhibition of DNA synthesis remains clearly recognizable even after 48 hr. On the other hand the antimetabolite cytosine arabinoside (ara-C) has a half life of about 15 hr and its inhibitory effect on the thymidine incorporation decreases accordingly.

A similar experiment has then been performed with 2,4,5-T. The half life of 2,4,5-T salts in rat serum has been reported to be about 3 hr (ERNE 1966). Our own measurements with 2,4,5-T-isooctylester in mice revealed an analogous figure. The rate of thymidine incorporation remained, however, at its acute exposure level for at least 24 hr and only after 48-72 hr became the values no more signficantly different from the untreated controls. In view of the relatively weak inhibition of DNA synthesis by 2,4,5-T this last result had to be expected anyway.

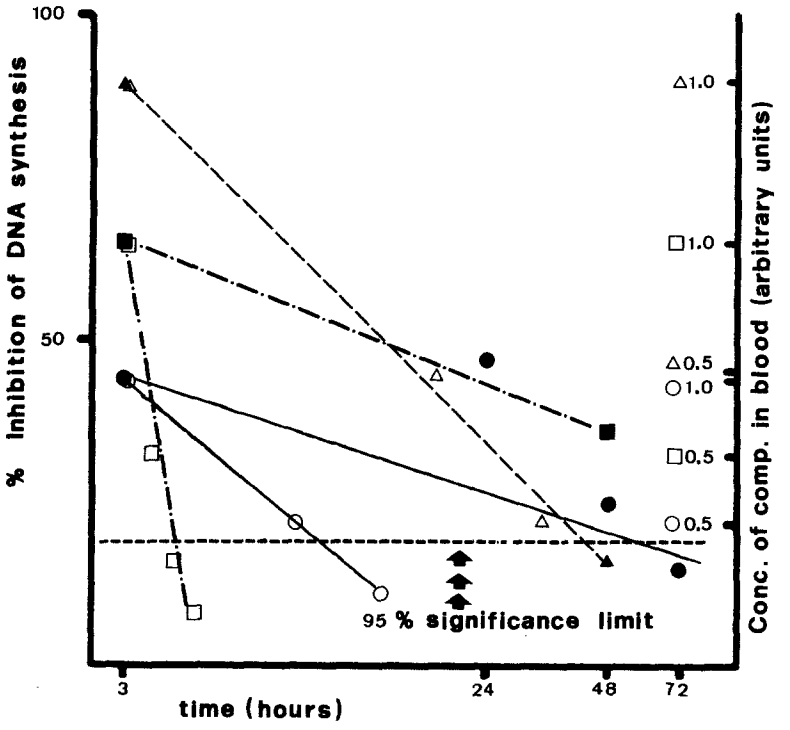

Figure 1. Time dependence of serum concentration (open symbols) and DNA synthesis inhibition (full symbols) of MMS (■—·—■), Ara-C (▲——▲) and 2,4,5-T-isooctylester (●—●).

A further clue as to the DNA damaging activity of phenoxyacids can be derived from the several reports published on mutagenic activity of such compounds in a variety of systems. It appears that the mutagenic activity of these substances is in the order MCPA > 2,4,5-T > 2,4-D which corresponds also to the order observed in our test system.

As of these substances only limited preliminary carcinogenicity data are available (HANSEN et al. 1971, INNES et al. 1969), the results obtained with the test described seem to call for more extensive investigations into the carcinogenic potential of these heavily used herbicides, the more so since very recently an increased incidence of sarcomas has been observed in Swedish spraymen (HARDELL 1977).

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