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GENOTOXICITY OF SELECTED HERBICIDES

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Summary. - *Twenty-two herbicides were studied in 67 tests for induction of DNA damage, gene mutation and chromosomal changes* in vitro *and* in vivo. *Triazine and urea-type herbicides were found to be inactive in all but one test. Of 4 thiocarbamates,* molinate *and* vernolate *caused chromosomal changes, namely increased incidence of sister chromatid exchanges and chromosomal aberrations* in vitro *and increased frequency of micronucleated polychromatic erythrocytes in mouse bone marrow. These compounds, however, did not cause gene mutation and only* molinate *gave equivocal positive result in bacterial repair test. Out of 11 miscellaneous herbicides,* ethofumesate, alachlor, dichlorprop *and* fluorodifen *proved to be positive only in one or two tests. In the light of clastogenicity of some thiocarbamates, serious consideration should be given to start animal carcinogenicity studies with these chemicals.*

Riassunto (Genotossicità di erbicidi selezionati). - *Sono stati studiati 22 erbicidi con 67 test per analizzare la loro capacità di induzione di danno al DNA, di mutazioni geniche e di alterazioni cromosomiche sia* in vitro *che* in vivo. *La triazina e erbicidi del tipo urea sono risultati negativi in tutti i test. Dei 4 tiocarbammati saggiati,* molinate *e* vernolate *hanno causato danno cromosomico, in particolare aumentata incidenza di scambi tra cromatidi fratelli e aberrazioni cromosomiche* in vitro, *e aumentata frequenza di eritrociti policromatici micronucleati nel midollo osseo di topo. Questi composti, tuttavia, non inducono mutazione genica e solo il* molinate *dà una chiara risposta positiva nel test di riparazione in batteri. Tra gli 11 erbicidi miscellanei,* ethofumesate, dahemid, alachlor, dichlorprop *e* fluorodifen *sono risultati positivi in uno o due test. Considerando la clastogenicità di alcuni tiocarbammati, sarebbe auspicabile che venissero condotti studi di cancerogenesi con questi agenti chimici.*

Introduction

Genotoxicity of agricultural chemicals has been a central issue of the toxicology in recent years. Several investigators studied a large number of pesticides for mutagenicity [1-4]. In 1984 we reviewed 83 pesticides for genotoxicity and carcinogenicity on the basis of the published data; many of them were, however, characterized for mutagenic activity mainly by the Ames test [5].

More recently, the interest for the genotoxic effect of pesticides has been shifted from quantitative studies to qualitative and comparative studies. Klopman *et al.* [6] reviewed the genetic activity of 54 pesticides on the basis of 5 *in vitro* tests assaying for gene mutation and DNA damaging activity. Unfortunately, only 5 compounds were tested in 5 assays and 25 in 4 tests, that is the survey is not comprehensive at all and included only *in vitro* tests.

Garrett *et al.* [7] evaluated the genetic profile of 65 pesticides in a more comprehensive system which included 670 *in vitro* and *in vivo* tests.

Although these studies analysed a large number of chemicals, it is a fair assumption that there are even more, publicly not available data. Based on the Pesticide Manual [8], we estimate the number of currently used or at least registered agricultural chemicals to be 560-600. The number of pesticides, currently being registered and partly used in Hungary is 230. Even this small number is much higher than the number of well studied pesticides, therefore a more thorough study of pesticides seems to be justified.

The issue, at what extent man-made chemicals contribute to human carcinogenic risk, is much debated. We think the large number of people exposed directly or indirectly to these chemicals justify the thorough study of these chemicals.

In this paper a brief summary of genotoxicological studies, carried out on selected herbicides at the Department of Morphology, National Institute of Hygiene, Budapest, is presented.

Materials and methods

The battery of short-term tests includes assays for DNA damaging effect, (*Escherichia coli (E. coli)* repair [differential killing] test, somatic cell mutation and recombination, (*Drosophila melanogaster,* wing hair mosaic test), gene mutation in prokaryotes (Salmonella/mammalian

microsome, Ames test) and in mammalian cells (CHO/HGPRT), chromosome damaging effect *in vitro* (CHO cells) and *in vivo* (mice).

The studies were carried out according to internationally accepted guidelines and recommendations, mainly according to the UKEMS guidelines [9]. Most of the tests were repeated at least once, with the exception of assays in *Drosophila*. *In vitro* assays were done in the absence and presence of metabolic activation system, using liver homogenate from aroclor-induced male Wistar rats and cofactors. Long-term carcinogenicity bioassays was done using inbred Fisher F344 rats.

Most of the chemicals were of technical grade purity, supplied by different Hungarian companies.

Results

Triazine herbicides

Of the numerous triazine herbicides, we have studied *atrazine, proglinazine, eglinazine* and *terbutryne* (Table 1). Studies with *simazine* is currently being done in our laboratory.

There are numerous, mostly conflicting data on short-term tests with *atrazine* [3, 10, 11]. It was generally found to be negative in most tests in prokaryotes *in vitro* either with or without mammalian microsome system, we obtained negative results with Salmonella/microsome assay, too. Microsome from various plants, however, was able to activate *atrazine* [3, 10]. When tested *in vivo*, it was found to induce dominant lethal mutation in mice and was positive in micronucleus test [12]. In fact, we repeated this test and found no increased incidence of micronucleated erythrocyte frequency in mice even at very high dose level (Table 1).

We have recently completed a long-term bioassay with *atrazine* in rats and statistically significant increased incidence of benign mammary gland tumours in males, that of uterine carcinomas in females, furthermore a dose-dependent, but non-significant increase of leukaemias/lymphomas were observed. *Atrazine* was tested in mice by oral application and was found not to induce tumors, the experiment, however, suffers from limitations [13]. We have knowledge of an unreported study, in which *atrazine* was found to induce malignant mammary gland tumours in female rats. Therefore, suggestive evidence exists for the carcinogenicity of *atrazine* in rats.

Out of the many structure-analogues, the other well-studied triazine is *simazine*. It was found to cause point mutation in mammalian cells and recessive lethals in *Drosophila*; negative results were obtained in several tests for DNA damaging effect, gene mutation and chromosomal changes [6]. There is an old, regarded at that time as preliminary carcinogenicity study in mice which was negative [13]. We are currently doing long-term bioassay in Fischer rats with *simazine*.

Eglinazine and *proglinazine* are original Hungarian molecules. Both proved to be negative for DNA damage, gene mutation and chromosome alteration (Table 1). *Terbutryne* rendered equivocal positive results when tested in *E. coli* for repairable DNA damage, but did not induce chromosome aberrations *in vitro* and was negative in micronucleus test (Table 1).

There are mostly negative studies, published on several other triazine herbicides in Salmonella [10].

The list of triazines is by no means complete, there are many compounds being used [8] but no genotoxicological data are available.

Because of the suggestive evidence for carcinogenicity and the stable nature of the triazines - despite the mostly negative short-term data - it is reasonable to consider possible restriction of use of these compounds.

Thiocarbamate herbicides

Of the other important group of herbicides, thiocarbamates, we studied *butylate, molinate, vernolate* and *eptam* (EPTC) (Table 2). In bacterial repair test, one positive (*molinate*) and one negative (*butylate*) result was obtai-

Table 1. - *Genetic activity of triazine herbicides*

	REP	MOS	SAL	SRL	HGP	SCE	CYT	MN	CCG
Atrazine 2-chloro-4-isopropylamino-6-methoxy-1,3,5-triazine			-					-	+
Simazine 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine		-							
Proglinazine N-(4-chlro-6-isopropylamino-1,3,5-triazin-2-yl)glycine		-	-					-	
Eglinazine N-(4-chloro-6-ethylamino-1,3,5-triazin-2-yl)glycine		-	-					-	
Terbutryne 2-ethylamino-4-methylthio-6-ter-buthylamino-1,3,5-triazine		+/-					-	-	

ried. The studied compounds invariable proved to be negative in Salmonella. In CHO/HGPRT system, *molinate* was negative, the other three chemicals are under testing in this system.

In mouse bone marrow micronucleus test, three of them proved to be positive. *Butylate* and *vernolate* produced statistically significant as compared to concurrent controls but not very high incidence (less than 0.5%) of micronucleated polychromatic erythrocytes. *Molinate*, however, was strongly positive, producing dose-dependent, reproducible increased incidence of micronucleated erythrocytes in the mouse bone marrow. *In vitro*, both *molinate* and *vernolate* caused structural aberrations and an increased incidence of sister chromatid exchanges in CHO cells in the presence of metabolic activation system. In the case of *vernolate*, the clastogenic effect was more pronounced - we observed numerous structural aberrations - the number of exchanges, however, were not as high as we expected.

Without activation, these thiocarbamates proved to be inactive.

There are ample information about the genotoxicity of chloroallyl-analogue thiocarbamates (*diallate, triallate, sulfallate*)[6, 7], few studies are, however, available for other thiocarbamates. *Molinate* was described (unpublished, personal communication) as inactive in prokaryotes, low mutagenic activity was found in the L5178 TK +/- system and it was found to be negative for chromosome aberration *in vitro* and in the micronucleus test. The data, however, were inadequate for proper evaluation. *Butylate* and *vernolate* was described to induce dominant and recessive lethal mutation in *Drosophila melanogaster* [14].

We studied EPTC only in Salmonella and found to be negative. According to published data, the compound is genetically inactive in several tests [3]. Most remarkably, no data were found concerning chromosomal effect of *eptam*. We are currently assaying *eptam* for cytogenetic effect.

In fact, the compounds we have tested, were of technical grade purity and repeated studies with analytically pure materials are in progress. Since the potential human exposure and the risk is due to the technical grade substances, our feeling is that this group of chemicals deserve more attention. *Molinate* is used mainly on rice fields, but the potential contamination of surface waters is not negligable. Out of the three structurally similar thiocarbamates, *molinate* was the most potent genotoxic agent, *vernolate* had only slight clastogenic effects. Considering the chemical structure of these compounds, it is difficult to explain this activity. Some carbamate pesticides were shown to produce aneuploidy due to the effect on the mitotic apparatus [15]. In the case of these alkylthiocarbamates, however, definite clastogenicity was found. A role of impurities can be ruled out with reasonable certainity, since very pronounced effect was observed *in vivo* in the case of *molinate*. On the other hand, the clastogenicity and SCE-inducing effect of these compounds were demonstrated at rather low doses.

Urea-type herbicides

In general, relatively few studies were carried out on urea-type herbicides. Grutman *et al.* [10] who reviewed the genotoxic activity of herbicides in prokaryotes found only 4 out of 15 ureas to be mutagenic. *Monuron* was reported to be clastogenic *in vitro* and *in vivo*. There is some evidence for carcinogenicity of *monuron* to experimental animals, but only in rats [13]. No other carcinogenicity data were available for other urea-type herbicides.

We studied three urea-type herbicides for genotoxicity. Similar to the published data [10], *metobromuron, chlorbromuron, diuron* were found mostly inactive (Table 3), only *chlorbromuron* caused increased incidence od sister chromatid exchanges in CHO cells. This effects was regarded as equivocal positive, since the increase did not reach the doubled value of the untreated controls and no dose-dependecy could be established.

Table 2. - *Genetic activity of some alkylthiocarbamates*

	REP	MOS	SAL	SRL	HGP	SCE	CYT	MNT
Butylate s-ethyl-di(isobutyl)thio-carbamate	-		-	-		-	-	+
Molinate s-ethyl-N,N-hexamethylene-thiocarbamate	+/-		-		-	+	+	+
Vernolate s-propyl-dipropyl- thiocarbamate			-			+	+	+
Eptam (EPTC) s-ethyl-dipropyl-thiocarbamate			-					

Table 3. - *Genetic activity of some urea-type herbicides*

	REP	MOS	SAL	SLR	HGP	CYT	SCE	MNT
Metobromuron 3-(H-bromophenyl)-l-methoxy-l-methylurea			-			-		-
Chlorbromuron 3-(H-bromo-3-chlorophenyl)-l-methoxy-l-methylurea			-				-	-
Diuron 3-(3,4-dichlorophenyl)-l,l-dimethylurea			-					

Table 4. - *Genetic activity of miscellaneous herbicides*

	REP	MOS	SAL	SLR	HGP	CYT	SCE	MNT
Alachlor 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide	+		-	-	-	+		-
Metolachlor alpha-chloro-6'-ethyl-N-(2-methoxy-l-methyl-ethyl)acet-o-toluidine			-					
Ethofumesate 2-ethoxy-2,3-dihydro-3,8-dimethylbenzofuran-5-yl-methanesulfonate	-	-	-	+	-	+	-	
Dahemid 2,2-dichloro-acetyl-hexamethylene imine	+/-	-	-		-		-	
Dichlorprop (+/-)-2-(2,4-dichloro-phenoxy)propionic acid	+		-			-		
Fluorodifen 4-nitrophenyl alpha,alpha,- alpha-trifluoromethyl-phenyl-etherether			+		-	-	-	

Miscellaneous pesticides

Most of the herbicides, studied by us belong to different chemical classes (Table 4). We have extensively assayed *alachlor* and found - similar to the literature [16] - in most tests negative. It induced, however, repairable DNA damage in *E. coli*, and caused chromosomal aberrations *in vitro*. *Alachlor* was tested in several experiments for carcinogenicity and although the tests were regarded as inadequate in some respects, they suggest carcinogenic activity [16].

We obtained contradictory data with *ethofumesate*, which proved to be negative almost in all tests, except the *Drosophila* sex-linked recessive lethal assay and SCE test *in vitro*. In SCE test the evidence for positivity was convincingly strong, borderline positivity was however, found in SLRL tes. *Fluorodifen* proved to be consistently positive in Salmonella, even using analytical grade substance. It was negative in the CHO/HGPRT system and it did not induce somatic cell mutation and recombination in *Drosophila* and it was inactive in the SCE test. Considering the only positive result in Salmonella, the genotoxic risk of *fluorodifen* is estimated as low.

The phenoxypropionic acid herbicide, *dichlorprop* induced somatic cell recombination in *Drosophila*, but it did not cause point mutation in Salmonella and showed no SCE-inducing ability.

Several other herbicides were assayed only in one or tests, therefore they are referred only in the summerizing table (Table 5).

Summing up our data, it can be stated, that the majority of the tests rendered negative results. The only consequent clustering of positive tests were obtained with thiocarbamates and namely with chromosomal effect.

Although our data are not sufficient enough to draw a farreaching conclusion, the main points can be summerized as follows:

a) triazine herbicides - similar to other pubished data - were found mostly negative in short term tests when assayed directly or in the presence of mammalian micro-

Table 5. - *Summary of genotoxicological tests carried out in our laboratory*

	REP	MOS	SAL	SRL	HGP	SCT	CYT	MNT
Triazines								
Atrazine			-					-
Eglinazine	-		-					-
Proglinazine	-		-					-
Terbutryn	+/-		-					-
Thiocarbamates								
Butylate	-		-	-	-	-	-	-
Molinate	+/-		-			+	+	+
Vernolate						+	+	+
EPTC			-					
Ureas								
Metobromuron			-				-	-
Chlorbromuron			-			-		-
Diuron			-					
Others								
Terbacil			-			-		
Ethofumesate	-	-	-	+		+	-	-
Dahemid	+/-	-	-	-		-	-	-
Alachlor	+		-	-		-	+	-
Metolachlor			-					
Dicamba			-					
Difenamid			-					
Dichlorprop		+	-			-		
Fluorodifen			+		-	-		
Chloridazon			-					
Trifluralin			-					

some, some triazines were however, shown to be activated by plant microsome preparations. Genotoxicity of triazine pesticides to mammalian cells and mammals is still not proven. Positive data for animal carcinogenicity raise the question of possible restriction of use of these chemicals;

b) some alkyl-thiocarbamates demonstrated pronounced chromosome-damaging effect all required metabolic activation *in vitro* and the most potent was the hexamethylene-derivate thiocarbamate, *molinate*. It seems to be rather important to carry out long-term animal bioassays with thiocarbamates, in order to asses human carcinogenic risk;

c) caution should be exercised to assess the predictive value of short-term tests of pesticides for animal carcinogenicity. Proper consideration should, however, be given to single positive results.

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