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STUDIES OF PESTICIDES MUTAGENICITY[1]

JADWIGA SZARAPIŃSKA-KWASZEWSKA, JERZY MIKUCKI, EDMUND BAKUNIAK, BOŻENA DUDKIEWICZ, EWA NAWROT, MARIA SOBIŚ[2]

Department of Pharmaceutical Microbiology, Institute of Pathology, Academy of Medicine, Łódź; Department of Pesticide Application, Institute of Organic Chemistry Industry, Warszawa

Summary. For the purpose of eliminating carcinogens from natural environment of human the mutagenicity of 21 new compounds-potential pesticides and that of 4 pesticides in common use was studied. Bacterial Ames'test using auxotrophic *S. typhimurium his*⁻ mutants with metabolic activation by Aroclor-induced rat liver microsomal enzymes was employed. Out of 25 studied substances 5 appeared to be mutagenic after metabolic activation. They were the following fungicides: 6-azauracil, monoethanolamine salt with 6-azauracil, technical olein amine salt with 6-azauracil, diethanolamine salt with 6-azauracil and N-methyl-N'decyl-thiomethyl-imidazolyl chloride. All of these preparations required metabolic activation.

The risk of human contact with pesticides is very large in view of their widespread application and a probability of food contamination. Many preparations used in agriculture have not only toxic properties, but may be a source of neoplasma in people and animals (Capurro 1980, Kimbrough 1979, Reuber 1980, Saleh 1980).

Most of compounds with proved carcinogenicity are capable to induce mutations, which permits to treat all mutagenic substances as potentially carcinogenic (Ames et al. 1973, 1979, Andrews et al. 1978, McCann et al. 1975, Rashid 1979, de Serres 1975).

In studies on mutagenicity of chemical compounds bacterial tests are commonly employed as short-term and cheap method. Out of them Ames'test (McCann et al. 1975) is characterized by the highest degree of correlation (90%) between mutagenicity and carcinogenicity.

Studies described in the present paper were aimed at detection of mutagenicity of 25 compounds with pesticidal properties including 4 used preparations and 21 new compounds.

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[2] First Author: M. D. Second Author: Docent M. D. Third Author: Prof. dr. hab. Fourth, fifth and sixth Authors: M. Pharm.

Present address: ul. Nowotki 137, 90-235 Łódź, Poland. Third Author: ul. Annopol 8, 03-236 Warszawa, Poland.

MATERIAL AND METHODS

STUDIED COMPOUNDS

An object of the studies were the following pesticide preparations received from the Institute of Organic Chemistry Industry in Warsaw: Fungicides having the structure of quaternary ammonium salts: two compounds from the group of β-methyl-β--nitro-vinyl-benzylammonium chlorides (IPO 2435 and IPO 2442), formyl-benzyl-ammonium choride (IPO 2504), two compounds from the group of alkoxymethylene--morpholine and piperidine chlorides (IPO 2507 and IPO 2567) as well as dimethylmorpholine chloride referred to growth substances (RW-3); organophosphorus insecticides: phosphate O,O-dimethyl-(1)-(2,5 dichlorophenyl)-2-chloro-2-carboxy(vinyl) (IPO 1391), phosphate O,O,dimethyl-O-(1-)2,4-dichlorophenyl(-2-bromo-2carboxy)vinyl (IPO 1401) and chlorfenvinphos, bromfenvinphos and methylbromfenvinphos; pesticides — derivatives of imidazole and benzimidazole — fungicides: N-methyl-N′-decyl-thiomethyl-imidazolyl chloride (IPO 4580) and carbendazim and a herbicide: 3-octyl-thiomethyl-1-methyl-imidazolyl chloride (IPO 3811); fungicides — derivatives of 6-azauracil: 6-azauracil (IPO 3834), monoethanolamine salt with 6-azauracil (IPO 3835), technical olein amine salt with 6-azauracil (IPO 3836) and dietanolamine salt with 6-azauracil (IPO 3837); herbicides — derivatives of urea: N-methyl-N (2-hydroxy)ethyl-N′-phenylurea (IPO 2363), N-(2-hydroxyethyl)-N-methyl-N′(3,4 dichlorophenyl)urea (IPO 3102), N-phenyl-N′-methylurea (IPO 4328); in addition to that fungicide — 2,3-dicyano-5,6-dihydro-1,4-ditiine (IPO 3013); herbicides: chloride of tridecyl-triethylglycine ester (IPO 3627) and hydrochloride of 6-chloro-3-nitro-2-propyl-aminobenzoic acid (IPO 3931) and one compound of bactericidal action — semicarbazone of glyoxalic acid (IPO 3884).

STUDY OF MUTAGENIC ACTION

Solutions of compounds for the investigations were prepared in solvents appropriate to them (water, dimethylsulphoxide, ethyl alcohol). The bacterial test according to Ames' (Ames et al. 1975) using reversion of auxotropic mutants *his*⁻ *Salmonella typhimurium*— strains: TA 1535, TA 100, TA 1537, TA 1538, TA 98 was employed. The mentioned strains were received from Prof. B. N. Ames, California University, USA.

The plate method was used. Solutions of the studied compounds in corresponding amounts were added to top-agar. We also used metabolic activation of the investigated compounds using a microsomal fraction (S-9) of rat liver (6-week males of the line Wistar), which were previously given Aroclor 1254 for enzyme induction.

RESULTS AND DISCUSSION

Most of the studied compounds displayed to various degree bactericidal action for the strains used for testing, and for that reason the range of the studied concentrations differed depending on particular preparations.

The largest amount of a compound added to a plate was 1000 μg, the lowest — 0.5 μg. Table 1, presenting the obtained results, contains the highest concentration of a compound, at which the largest number of revertant colonies occurred. A criterium of mutagenic action of a compound in Ames'test is the obtaining of a number of revertant colonies two-fold higher in the presence of a compound as compared to

Table 1. Mutagenic action of potential pesticides — direct and with metabolic activation (S-9) on auxotrophic *S. typhimurium his*⁻ mutants

Compound	Amount of compound per plate in μg	Number of *his*⁻ revertant colonies/plate									
		TA 1535		TA 100		TA 1537		TA 1538		TA 98	
		—	S-9	—	S-9	—	S-9	—	S-9	—	S-9
Fungicides of quaternary ammosium salt structure											
Control	0	17	12	126	115	5	7	16	—	24	34
IPO 2435	2	16	13	101	86	7	7	21	—	19	40
IPO 2442	5	20	10	146	103	6	11	21	—	19	49
IPO 2504	10	23	11	118	93	7	6	17	—	23	24
IPO 2507	20	16	14	138	98	6	7	30	—	37	39
IPO 2567	5	22	9	120	109	5	7	25	—	25	30
Growth agent RW-3	100	24	13	147	75	4	5	24	—	29	49
Organophosphorus insecticides											
Control	0	12	13	70	74	12	16	14	40	15	54
IPO 1391	100	6	16	54	67	9	11	19	46	15	49
IPO 1401	50	10	9	78	88	7	19	19	48	18	45
Chlorfenvinphos	100	9	11	53	44	6	19	12	42	10	45
Bromfenvinphos	500	13	17	42	38	6	15	16	48	21	37
Methylbromfenvinphos	50	11	15	48	47	8	10	15	39	16	44
Pesticides derivatives of imidazole and benzimidazole											
Control	0	12	13	70	74	12	16	14	40	15	54
IPO 4580	20	17	216	69	411	8	12	18	36	22	34
Carbendazim	1000	18	11	35	46	6	16	10	53	16	41
IPO 3811	100	13	11	73	90	6	7	15	44	17	38
Herbicides derivatives of urea											
Control	0	12	13	70	74	12	16	14	40	15	54
IPO 2363	1000	10	12	74	60	8	17	14	56	14	37
IPO 3102	200	8	10	75	68	9	16	17	49	10	50
IPO 4328	1000	16	8	59	42	6	14	9	58	15	46
Fungicides derivatives of 6-azauracil											
Control	0	12	13	92	97	8	16	16	40	18	47
IPO 3834	10	12	8	80	122	8	9	13	452	15	328
IPO 3835	10	13	14	82	110	9	16	15	256	17	90
IPO 3836	10	13	9	81	90	7	7	17	437	19	278
IPO 3837	100	11	12	90	125	9	18	14	358	15	263
Pesticides of various structure											
Control	0	12	13	92	97	8	16	16	40	18	47
IPO 3013	200	11	9	73	66	4	14	9	61	11	41
IPO 3627	5	14	18	89	87	8	4	15	38	18	46
IPO 3931	1000	10	6	93	67	11	19	22	57	11	46
Bactericide											
Control	0	12	13	92	97	8	16	16	40	18	47
IPO 3884	100	12	14	76	56	13	18	10	50	15	41

the number of spontaneous revertant colonies grown in the control. As seen from the presented data, none of the obtained preparations caused reverse mutation of the test strains during direct investigation (without metabolic activation). In the presence of microsomal fraction (S-9) five compounds displayed mutagenic action to various test strains. All 4 fungicides — derivatives of 6-azauracil (IPO 3834, IPO 3835, IPO 3836, IPO 3837) appeared to be mutagens for two strains — TA 1538 and TA 98. The mutagenic activity was pronounced there (5-10-fold number of induced revertant colonies in comparison with spontaneous ones) and nearly similar for all compounds except IPO 3835, which was mutagenic only for the strain TA 1538. Fungicide IPO 4580 (N-methyl-N'-decyl-thiomethyl-imidazolyl chloride) after metabolic activation became mutagenic for the strains TA 1535 and TA 100. In the so-far studies using Ames'test and other short-term microbial tests it was found that many common applied pesticides (Marshall et al. 1976, Sakai et al. 1978, Sandhu et al. 1980, Shirasu et al. 1976, Tezuka et al. 1980, Usha Rani et al. 1980, Waters et al. 1980), as well as decay products of some pesticides (Rashid 1979) have mutagenic properties.

Results of earlier studies on pesticides and many other chemicals at various applications showed that carcinogenic and mutagenic action of these compounds is associated to a large degree with the chemical structure of a compound. Mutagenic action of some organophosphorus pesticides has been proved (Hanna et al. 1975, Shirasu et al. 1976, Wild 1975). In the present studies none of the obtained organophosphorus derivatives appeared to be mutagenic. In the earlier studies (Szarapińska-Kwaszewska et al. 1982) 12 other organophosphorus pesticides did not display mutagenicity of none of these compounds. Well-known is mutagenicity and carcinogenicity of urea derivatives including pesticides of the same structure (Zimmer et al. 1976). In the present studies none of the preparations having such grouping in the molecular appeared to be mutagenic.

From the literature data it follows that compounds with a very similar structure are often found to have different properties depending on details of their molecular structure (Rao et al. 1978, Sakai et al. 1978, Takeda et al. 1980), and, therefore, even close structural similarity of a given compound to known mutagens and carcinogens does not determine its carcinogenicity or mutagenicity.

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BADANIE MUTAGENNOŚCI PESTYCYDÓW

Streszczenie

W celu eliminacji kancerogenów ze środowiska naturalnego człowieka badano mutagenność 21 nowych związków — potencjalnych pestycydów oraz 4 pestycydów już stosowanych. Zastosowano bakteryjny test Amesa wykorzystujący auksotroficzne mutanty *S. typhimurium his*⁻ przy udziale metabolicznej aktywacji enzymami mikrosomalnymi wątroby szczurów indukowanych Aroclorem. Spośród 25 badanych substancji 5 wykazało działanie mutagenne po aktywacji metabolicznej. Były to fungicydy: 6-azauracyl, sól 6-azauracylu z monoetanoloaminą, sól 6-azauracylu z aminą oleinową techniczną i sól 6-azauracylu z dwuetanoloaminą oraz chlorek N-metylo-N′-decylotiometylo-imidazoliniowy.

ИССЛЕДОВАНИЕ МУТАГЕННОСТИ ПЕСТИЦИДОВ

Резюме

С целью элиминации канцерогенов из естественной среды человека исследовалась мутагенность 21 новых химических соединений — потенциальных пестицидов и 4 пестицидов, уже применяемых в практике. Был применён бактерийный тест Амеса, использующий ауксотрофические мутанты *S. typhimurium his*⁻ с участием метаболической активации микросомальными ферментами печени крыс, индуцированных Ароклором. Из 25 исследованных субстанций 5 обнаружило мутагенное действие по метаболической активации. Это были фунгициды: 6-азаурацил, соль 6-азауралила с моноэтаноламином, соль 6-азаурацила с амином олеиновым(техническим), соль 6-азаурацила с диэтаноламином и хлорид N-метил-N′-децилтиометил-имидазолина.