Mutagenicity Testing of Cyclamate and Some Pesticides in *Drosophila melanogaster*

In the course of a research program, 12 pesticides including 4 related compounds and 1 sweetening agent with 2 suspected metabolites, were tested on *Drosophila*. In order to measure possible genetic effects of the compounds selected, sex-linked lethal tests were performed by feeding adult males.

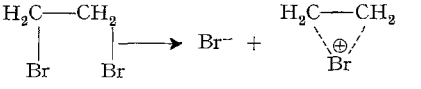
The test substances were administered in solution or as fine suspensions by treating 2-day-old adult Berlin K males for 3 days[1]. Compounds suggested to be insoluble in water were completely dissolved in DMSO or ethanol first and then diluted with 5% sucrose solution to reduce the DMSO or ethanol content down to 2 and 5%, respectively, and to yield a homogenous solution. Depending on the solubility of the compounds under weakly alkaline or acidic conditions, a m/15 phosphate buffer were used to keep the pH constant at 6.8 or 8.0 (listed in the Tables for every compound). After treatment, the treated (control) males were mated to two In (1) sc^{s1L} sc^{s8R} + S, sc^{s1} sc^{s} w^{a} B females (genetic symbols see ref. [2]) for 3 days. A sequence of two 3-day brood periods was initiated followed by one 4-day brood. At the end of each breeding period, the treated male was transformed to a new vial and remated with 2 new females.

Except with pentachlorophenol and cyclohexanoneoxime, replicates were carried out using different concentrations. However, when the compound tested was ineffective, only the mean values are listed in the Table.

The data in Table I for 2.4-D and chemically related compounds show that MCPA may be considereed a weak mutagen in *Drosophila*. When the data from all MCPA-experiments are pooled and compared to the corresponding control (control 2), this difference in the x^2-test is on the verge of significance, $P = 0.015$. 2.4-D, 2.4.5-T and MCPB failed to induce a noticeable rise of recessive lethal frequencies; however, there might be a very weak effect for MCPB at 4.4 mM as well as 8.8 mM. A considerable decline in fertility resulted at 7.2 mM 2.4.5-T, as expressed by the reduced number of chromosomes tested in broods 2 and 3. The sterilizing effect might originate from similar cytogenetic effects as observed for 2.4.5-T after female treatment[3].

As indicated in Table II, 2 of 8 compounds, namely 1.2-dibromoethane and 1.2-dibromopropane, clearly show mutagenic activity in *Drosophila*. Differing from the generally observed high sensitivity of mature sperm to chemical mutagens, obviously spermatids and spermatocytes (corresponding to broods 2 and 3) are more affected by the halogenalkanes than mature sperm; 1.2-dibromoethane was suggested to be mutagenic in *Neurospora crassa*[4] and the Host-mediated Assay[5]. The closely related 1.2-dichloroethane is a potent mutagen in *Drosophila*[6]. 1.1-dibromoethane, ineffective in *Drosophila*, induces transversions and transitions[7] on phage S13.

It is well-known that vicinal 1.2-dibromides readily react to form highly unstable bromonium ions in solution[8].



The bromonium ions can be considered as 'biological alkylating agents' which will alkylate cellular nucleophiles, including DNA. The initial product of alkylation of a hetero-atom such as O, N, or S would be the 2-Bromoethyl-derivatives, which would be a 'half-mustard' type reagent capable of another alkylation reaction. Thus, 1.2-dibromoethane can be considered as a bi-functional alkylating agent capable of introducing cross-links into biological molecules. Another potent mutagen in *Droso-*

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[2] D. L. LINDSLEY and E. H. GRELL, *Genetic Variations of Drosophila melanogaster* (Carnegie Inst. Wash. Publ. 627, 1968), p. 472.
[3] L. DÄVRING and M. SUNNER, Hereditas *68*, 115 (1971).
[4] H. U. MALLING, Genetics *61*, Suppl. 2/2, 39 (1969).
[5] W. BUSELMAIER, R. RÖHRBORN and P. PROPPING, Biolog. Zentblatt *91*, 311 (1972).
[6] V. F. SHAKARNIS, Genetica *5*, 89 (1969).
[7] I. TESSMANN, EMS-Newslet. *4*, 33 (1971).
[8] E. S. GOULD, *Mechanism and Structure in Organic Chemistry* (Holt, Rinehart and Winston, New York 1965), p. 561.

Table I. Incidence of *X*-chromosomal recessive lethals in meiotic and postmeiotic male germ cells of *Drosophila* treated by herbicides

Test Substance	Concentration (mM)	pH	Brood 1 lethals/ chromos.	%	Brood 2 lethals/ chromos.	%	Brood 3 lethals/ chromos.	%	1–3 lethals- chromos.	%
Control 1	—	6.8	4/1633	0.24	3/1408	0.21	5/1402	0.36	12/4443	0.27
Control 2	—	8.0	1/1210	0.08	1/1112	0.09	2/1124	0.18	4/3446	0.12
2,4-D	4.5	6.8	1/617	0.16	1/598	0.17	0/594	—	2/1809	0.11
	9.0	6.8	0/413	—	0/413	—	2/400	0.50	2/1226	0.16
2,4,5-T, Na^+-salt	3.6	6.8	5/1827	0.27	2/617	0.32	2/620	0.32	9/3064	0.29
	7.2	6.8	2/567	0.35	0/49	—	0/285	—	2/901	0.22
MCPA	5.0	8.0	5/708	0.71	3/706	0.42	6/675	0.89		
	5.0	8.0	2/595	0.34	1/595	0.17	2/619	0.32		
	5.0	8.0	3/593	0.51	0/580	—	1/742	0.21		
	5.0	8.0	10/1896	0.53	4/1881	0.21	9/2036	0.44	23/5813	0.40
	10.0	8.0	7/1191	0.59	2/598	0.33	0/604	—	9/2393	0.38
MCPB	4.4	8.0	6/1159	0.52	1/567	0.18	1/613	0.16	8/2339	0.34
	8.8	8.0	4/1926	0.21	1/1108	0.09	5/1111	0.45	10/4145	0.24

2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCPA, 4-chloro-2-methylphenoxyacetic acid; MCPB, 4-(4-chloro-2-methylphenoxy)butyric acid.

Table II. Effects of some pesticides on recessive lethal frequencies in meiotic and postmeiotic male germ cells of *Drosophila*

Test Substance	Concentration (m*M*)	pH	Brood 1 lethals/ chromos.	%	Brood 2 lethals/ chromos.	%	Brood 3 lethals/ chromos.	%	1–3 lethals/ chromos.	%
Control 1	—	6.8	4/1633	0.24	3/1408	0.21	5/1402	0.36	12/4443	0.27
Control 3 [a]		6.8	5/2207	0.23	1/1817	0.06	3/1850	0.16	9/5874	0.15
Control 4 [b]		6.8	1/613	0.16	0/594	—	0/622	—	1/1829	0.05
Pentachlorophenol, sodium salt	7.0	6.8	0/609	—	0/618	—	2/597	0.34	2/1824	0.11
Pentachloro-nitrobenzene [c]	3.3 [a]	6.8	3/1207	0.25	2/1202	0.17	5/1150	0.43	10/3559	0.28
Folpet [c]	3.3 [b]	6.8	3/1198	0.25	2/1212	0.17	4/1229	0.33	9/3639	0.25
(N-(Trichloromethyl-thio) phthalimide)										
1-bromoethane	2.7	6.8	2/1186	0.17	3/920	0.33	1/920	0.11	6/3026	0.20
	8.2	6.8	6/923	0.65	1/615	0.16	1/615	0.16	8/2153	0.37
1,1-dibromo-ethane	2.7	6.8	4/1090	0.37	3/918	0.33	1/910	0.11	8/2918	0.28
	5.4	6.8	2/920	0.22	1/612	0.16	2/603	0.33	5/2135	0.23
1,2-dibromoethane	0.3	6.8	6/1201	0.50	18/1209	1.49	16/1234	1.30	40/3644	1.10
1,2-dibromopropane	5.0 [a]	6.8	4/602	0.66	5/619	0.81	9/622	1.45	18/1843	0.98
1,3-dibromopropane	2.7 [a]	6.8	4/1107	0.36	0/919	—	1/926	0.11	5/2953	0.17

[a] Test solution with 2% DMSO. [b] Test solution with 5% aethanol. [c] Tested as suspension.

phila, 1.2-dichloroethane, would be expected to undergo similar reactions. The 1.3-dibromopropane reacts very slowly in comparison with 1.2-dibromoethane, which probably explains why it failed to procedure any observable genetic effects under these experimental conditions.

Pentachlorophenol and pentachloronitrobenzene, the latter being reported to have mutagenic activity in *E. coli*[9], were negative in the sex-linked lethal test in *Drosophila*. Our data also reveal no evidence for a genetic activity of folpet in *Drosophila* and are in line with those of KRAMERS and KNAAP[10] for folpet.

Contradictonary results have been reported as regards a possible mutagenic action of the sweetener cyclamate and its metabolic product cyclohexylamine in mammals in vivo and in vitro[11–19]. In *Drosophila*, cyclamate and cyclohexylamine are not mutagenic, as indicated in Table III and reported by BROWNING[20], even when high doses such as 25.0 m*M* were applied. Negative results

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[20] L. S. BROWNING, Failure to detect mutagenicity by injection of cyclohexylamine on N-hydroxy-cyclohexylamine into *Drosophila*, Abstr. 2nd Ann. Meeting EMS (1971), p. 19.

Table III. Recessive lethal frequencies in meiotic and postmeiotic male germ cells of *Drosophila* following treatment by Na-cyclamate, cyclohexylamine and cyclohexanoneoxime

Test Substance	Concentration (m*M*)	pH	Brood 1 lethals/ chromos.	%	Brood 2 lethals/ chromos.	%	Brood 3 lethals/ chromos.	%	1–3 lethals/ chromos.	%
Control 1	—	6.8	4/1633	0.24	3/1408	0.21	5/1402	0.36	12/4443	0.27
Na-cyclamate	5.0	6.5–7.0	0/597	—	0/585	—	4/584	0.68	4/1766	0.23
	25.0	6.5–7.0	4/1202	0.33	2/1013	0.20	3/1021	0.29	9/3236	0.28
Cyclohexylamine	10.1	6.8	4/996	0.40	0/1011	—	1/995	0.10	5/3002	0.17
Cyclohexanone-oxime	8.8	6.8	0/622	—	0/593	—	3/603	0.50	3/1818	0.17

were obtained, too, with cyclohexanoneoxime, probably another product of cyclamate metabolism.

In summary, out of all compounds tested, 1.2-dibromoethane and 1.2-dibromopropane were found to be definitely mutagenic in *Drosophila*. The effects produced by MCPA and, to a lower extent, also MCPB, are not sufficient to decide whether these two compounds are also active in producing recessive lethals or not. At least MCPA seems to be a weak mutagen in *Drosophila*[21].

Zusammenfassung. 15 Substanzen, vorwiegend Pestizide, wurden an männlichen Keimzellen von *Drosophila* auf ihre genetische Wirksamkeit untersucht. Für 1.2-Dibromäthan, 1.2-Dibrompropan und MCPA konnte eine genetische Aktivität nachgewiesen werden. Halogenalkane zeigen eine klare Struktur-Wirkungsbeziehung.

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Tertiary Trisomics in *Pennisetum typhoides*

In tertiary trisomics the extra chromosome is translocated; the ends of extra chromosome are homologous with the ends of 2 different chromosomes. The diagnostic characteristics of tertiary trisomics are the formation of pentavalents (chain of 5 chromosomes or 2 ring bivalents linked by a translocated chromosome- 'dumb-bell' shape) and the absence of ring quadrivalents.

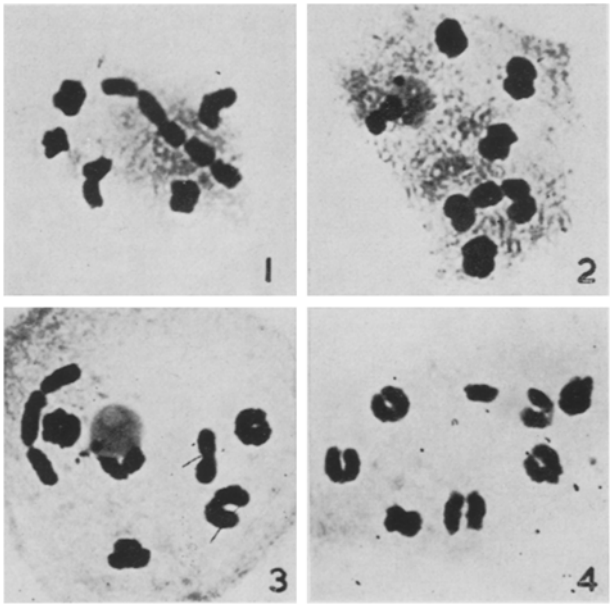

Fig. 1–4. Chromosome associations at diakinesis/metaphase I in tertiary trisomics. ×1300. 1. 1_V+5_{II} (chain-shaped pentavalent). 2. 1_V+5_{II} (dumbbell-shaped pentavalent). 3. $1_{III}+6_{II}$. 4. $7_{II}+1_I$.

Inspite of great usefulness of tertiary trisomics in assigning genes to a particular chromosome arm and the determination of centromere position, these have been produced only in *Datura*, corn, barley, tomato and rye. In tomato several genes have been located using tertiary trisomics[1]. The present communication reports for the first time the production of tertiary trisomics in pearl millet, *Pennisetum typhoides* (Burm.) S. & H. ($2n = 14$).

Several progenies of selfed interchange-heterozygotes and their crosses with different gene markers were examined. The off-type plants having short stature, thin stem and narrow leaves were marked. The PMC analysis of majority of thus selected plants revealed them to be trisomics. Besides interchange trisomics, 25 tertiary trisomics were isolated from a population of 5,000 plants.

On an average, the tertiary trisomics showed an association of 5 chromosomes at diakinesis/metaphase I in 15.0% of the cells. The pentavalents were either chain-shaped (Figure 1) or dumbbell-shaped (Figure 2). As seen in the Table, the modal configuration was $1_{III}+6_{II}$ (Figure 3) and the next most frequent class was $7_{II}+1_I$ (Figure 4). Chromosome associations of $1_{IV}+5_{II}+1_I$, $1_{III}+5_{II}+2_I$ and $6_{II}+3_I$ were rare, the frequency being 0.54, 1.36 and 1.09% respectively. The chromosomal distribution of 8–7 was predominant (95.1%), only a few cells showed laggards. Pollen fertility in these trisomics, as studied from mature anthers by staining with aceto-carmine, was reduced to 51.1 to 87.8% of the diploid.

The tertiary trisomics showed the characteristic features of trisomy in pearl millet. In contrast to the primary trisomics[2], the change in morphology of tertiary trisomics was not attributable to particular chromosomes.

[1] G. S. KHUSH and C. M. RICK, Can. J. Genet. Cytol. *9*, 610 (1967).

[2] B. S. GILL, S. S. VIRMANI and J. L. MINOCHA, Can. J. Genet. Cytol. *12*, 474 (1970).

Meiotic behaviour of tertiary trisomics in pearl millet, *Pennisetum typhoides*

	Chromosome association at diakinesis/metaphase I						Anaphase I distrib.	
Cells	1_V+5_{II}	$1_{IV}+5_{II}+1_I$	$1_{III}+6_{II}$	$1_{III}+5_{II}+2_I$	$7_{II}+1_I$	$6_{II}+3_I$	8-7	7-1-7
Number	55	2	187	5	144	4	233	12
Percent	14.99	0.54	50.95	1.36	31.06	1.09	95.10	4.90