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<https://doi.org/10.5109/23909>

出版情報：九州大学大学院農学研究院紀要. 34 (4), pp.421-425, 1990-03. Kyushu University

バージョン：

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Induction of Recessive Lethal Mutants by Dioxin and Dibenzofurans in *Drosophila melanogaster*

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(Received November 13, 1989)

The mutagenicity of the polluting chemicals 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin, 2, 3, 4, 7, 8-pentachloro-dibenzofuran and 1, 2, 3, 4, 7, 8-hexachloro-dibenzofuran was tested by using *Drosophila melanogaster*. After wild type spermatogenic males were fed with the chemicals and mated to Muller-5 females, sex-linked recessive lethal mutations occurring on the X-chromosome could be detected and new lines were established. However, the results bear the problem that the most frequent mutations were observed in the treatment at the stage of spermatogonia, rather than at the meiosis and maturation stages at which standard inheritable variation would be more frequently induced.

INTRODUCTION

Recent progress of chemical industries accompanies an accumulation of compounds that are scarcely degraded under the natural conditions. These stubborn residues include dioxins (polychlorinated dibenzo-*p*-dioxins, PCDDs) and polychlorinated dibenzofurans (PCDFs). PCDD and PCDF have many kinds of isomers, depending upon the number and position of chlorine atoms; some of them are highly toxic to a certain animals in a species-specific manner (Masuda, 1987a; Nishizumi, 1987; Masuda, 1988).

PCDDs and PCDFs not only occur as impurities of chlorine-containing industrial chemicals during production but also are easily produced by burning such substances during incineration, and thus have been widely spread in environments (Masuda, 1987a, b; Kashimoto, 1987; Masuda, 1988; Safe, 1989).

The toxicity of PCDDs and PCDFs to animals have been studied in terms of lethality, malformation, fetal toxicity, induction of aryl hydrocarbon hydroxylase activities, effects on DNA and so on (Nagayama et al., 1985; Nagayama, 1987; Nishizumi, 1987; Denison et al., 1988; Cuthill and Poellinger, 1988; Wahba et al., 1988). However, whether or not these chemicals have inheritable effects is uncertain; many conflicting results have been obtained for mutagenic potencies on mammals

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and other organisms including *Drosophila melanogaster* (Zimmering *et al.*, 1985 ; Giri, 1986 ; Safe, 1989). In view of the well-known advantages of *D. melanogaster* as an experimental material for genetics, we have tried to extend the investigation with this species. In the present study the spermatogenic male adults of *D. melanogaster* are treated with the chemicals to detect mutagenic potencies.

MATERIALS AND METHODS

As chemicals 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), 2, 3, 4, 7, 8-pentachloro-dibenzofuran (PCDF) and 1, 2, 3, 4, 7, 8-hexachloro-dibenzofuran (HCDF) were chosen. TCDD is a typical PCDD and known for its high lethality to the guinea pig, comparable to that of tetrodotoxin (cf. Masuda, 1987a). The other two are PCDFs. All were synthesized as described previously (Kuroki *et al.*, 1980). Their purity was more than 99% in terms of gas chromatography/mass spectrometry. These were dissolved in olive oil at a concentration of 40 $\mu\text{g/ml}$.

The Muller-5 strain of *D. melanogaster* was used as a detector for recessive, sex-linked mutants (Spencer and Stern, 1948) : The X-chromosome of the strain possessed w^a (white apricot) and *B* (**Bar**) as markers. This chromosome also has an inversion to prevent crossing-over. Wild type males of the Oregon-R strain one day after emergence were injected with the olive oil solution of the test chemical via a thin needle of glass capillary. Alternatively, the male flies were fasted for 24 hr and then reared for 24 hr with a diet made of honey plus yeast that was mixed with the olive oil solution of the chemical. The treated males were mated with Muller-5 females according to the schedule described in RESULTS and DISCUSSION. Then the F_1 progenies were sib-mated. When males with normal phenotype could not be found in the F_2 , there would be a lethal mutant on the X-chromosome and the *B* females in the F_2 were crossed with the Bw^a males to establish a mutant line. The survival rate of treated flies and their F_1 was more than 95%, not different from the control given the vehicle olive oil. All procedures were made at 22°C.

RESULTS AND DISCUSSION

Male Oregon-R flies were treated with TCDD, PCDF or HCDF and surveyed for the detection of the recessive, lethal mutants occurring on the X-chromosome by crossing with Muller-5 females. The first mating was begun on day 0, shortly after the treatment. The mated females were collected and replaced by new females on day 3 and then on day 6. The progenies obtained after these 3 types of mating were named, in that order, broods A, B and C. It takes about 9 days that sperm mature from the stem cells (Lindsley and Tokuyasu, 1980). Therefore, the approximate spermatogenetic stages at which the chemical caused effects could be assumed : the stage of sperm maturation for brood A, the stage of meiosis for brood B and the stage of somatic mitoses of the spermatogonia for brood C.

As shown in Table 1, injection of the chemicals to the flies gave one mutant, whereas oral administration produced 11 mutants. Thus the feeding method gave more marked results than the injection method. Previously, both injection and feeding of TCDD gave no lethal mutants (Zimmering *et al.*, 1985). In the present study, the dose

Table 1. Induction of mutations by TCDD, PCDF and HCDF."

Mode of administration	Chemical	Brood ^b				
		A	B	C	Total	%
Injection	TCDD	0/65	0/192	1/104	1/361	0.28
	PCDF	0/44	0/158	0/77	0/279	0.00
	HCDF	0/70	0/117	0/25	0/172	0.00
	Control ^c	0/79	0/161	0/45	0/285	0.00
Ingestion	TCDD	3/413	0/642	0/361	3/1,416	0.21
	PCDF	0/454	0/500	1/321	1/1,275	0.08
	HCDF	0/488	0/570	6/554	8/1,612	0.50
	Control ^c	0/418	0/532	2/341	2/1,291	0.15

^aData were given by the number of mutants per that of the F₁ pair assessed.

^bBroods were obtained by pairing on days 0 to 3, 3 to 6 and 6 to 9, respectively, after administration of the chemical to males. Thus broods A, B and C referred to as treatments at the spermatogenic stages of maturation, meiosis and spermatogonia, respectively.

Control flies received the solvent olive oil.

by injection was technically the maximum (0.2 μ l of the above olive oil solution per individual fly). As to the oral administration, on the other hand, 25 μ l of the test solution was introduced per one rearing bottle and mingled with the diet by which several male flies were reared, and thus the net ingested amount was unknown.

Brood C exhibited more clear-cut mutation rates than broods A and B. It should be noted here that the individuals of brood C may have received disturbance at the stage of spermatogonia as stated above. Generally the spermatogonial stage is more insensitive to mutagenic treatments than the other spermatogenesis stages probably because of the high repair capacity and elimination of cells during multiplication of spermatogonia (e. g. Yoshikawa et al., 1984; Shima and Shimada, 1988). Therefore, a group of mutants in brood C obtained here are assumed to be of a cluster that formed as a clone of a mutant spermatogonium.

The mutation rate for the control in the ingestion experiment (0.16%) will seem to be extremely high at first glance. However, this value is reasonable in case of *D. melanogaster* without any precaution to depress spontaneous mutations because of transposable elements (cf., e. g. Yoshikawa et al., 1984). At present, the percentages smaller than 0.16 were obtained for the PCDF-ingested flies and for most of the injected animals including the control. We infer that some unexpected conditions, e. g. selective survival of healthy individuals, might have disturbed the investigation system.

In spite of the above compromise, the mutants detected here could be established as lines. Formerly *D. melanogaster* was reported to be insensitive to TCDD in terms of mutagenesis (Zimmering et al., 1985) as well as of the potency of inducing aryl hydrocarbon hydroxylases (Bigelow et al., 1985; Hällström, 1986). In the present results a group of mutations were produced by HCDF (particularly in brood C), and also the potencies of TCDD and PCDF could not be ruled out, although the mutation rates seemed to be low if any. We are trying to increase further the dose of oral administration to the flies. Observation of developmental abnormality in *D. melanogaster* to detect primary effects of the chemicals, and application of the somatic

mutation test devised for *D. melanogaster* to detect genotoxic activity without rearing progenies (Graf *et al.*, 1984) are also under way in our laboratory and the results will be published elsewhere.

ACKNOWLEDGEMENTS

We thank Dr. C. Kiyohara of the Department of Public Health, Faculty of Medicine, Kyushu University, for advice and help about the treatment of chemicals. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. 62602015) from the Ministry of Education, Science and Culture of Japan.

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