Environmental and Experimental Botany, Vol. 29, No. 2, pp. 237–240, 1989.
Printed in Great Britain.

0098-8472/89 $3.00 + 0.00

MUTAGENICITY OF ATRAZINE IN *SCHIZOSACCHAROMYCES POMBE* LINDNER WITH AND WITHOUT METABOLIC ACTIVATION BY MAIZE

M. MATHIAS, J. GILOT-DELHALLE and J. MOUTSCHEN

Laboratory of Genetic Toxicology, Department of Botany, University of Liège, Sart-Tilman B22, B-4000 Liège, Belgium

(*Received* 23 *December* 1987; *accepted in revised form* 27 *July* 1988)

MATHIAS M., GILOT-DELHALLE J. and MOUTSCHEN J. *Mutagenicity of atrazine in* Schizosaccharomyces pombe *Lindner with and without metabolic activation by maize.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **29,** 237–240, 1989.—The mutagenic activity of two preparations of the herbicide atrazine (pure and a commercial formulation) was tested on yeast *Schizosaccharomyces pombe* (*ade 7-C8*) with and without *in vivo* activation by maize. The mutagenicity was much higher after activation (S1 fraction). The commercial formulation showed far higher activity than the pure compound. The genotoxic efficiency of a maize chloroplastic fraction after activation was also much higher than that of the supernatant, suggesting a transformation of the promutagen into an active mutagen in the organelle.
Key words: Mutagenicity, atrazine, *Schizosaccharomyces pombe*, yeast.

INTRODUCTION

ATRAZINE is a selective herbicide of the *s*-triazine group which has been widely utilized in agriculture. Atrazine's toxicity and, in particular, its genotoxicity has been investigated in various test systems and yielded contradictory results. An increase in chromosome damage was induced in mitotic cells of *Hordeum*[22,23] and *Vicia*,[24] in natural plant populations sprayed with the herbicide,[24] and in mitotic and meiotic chromosomes of *Sorghum*.[6–9] One study reported no induction of chromosome damage by atrazine in plant cells.[12] Point mutations were induced in *Hordeum*[23] and *Zea*.[14,17] Even at doses higher than those used for field spraying, no significant chromosome damage could be induced in mice after intra-peritoneal injections of atrazine.[3]

In microorganisms, atrazine has yielded contradictory results. The mutagenic efficiency was low in *Salmonella typhimurium* and phage T4,[1] but was higher in *Bacillus subtilis* and *Escherichia coli*.[20] No mutagenic effects were observed in *Saccharomyces cerevisiae* (strain D4).[21]

In 1975, it was observed that triazines, including atrazine, are promutagenic substances activated by plant tissues into mutagens.[13–15,18] Treatments of *Saccharomyces cerevisiae* by S1 fractions of maize previously treated with atrazine increased gene conversion[13,14,16] and also mutation rate.[10]

In the majority of the examples mentioned above, the commercial formulation of atrazine is not described. Therefore, we have compared the mutgenic and clastogenic efficiencies of pure atrazine with a commercial formulation. The latter showed a higher activity in a mutation test in *Schizosaccharomyces pombe* and in a test of clastogenicity in *Nigella damascena*.[11]

The present experiments were designed to compare the effects of pure and commercial atrazine on *Schizosaccharomyces pombe* after *in vivo* activation by maize. Since atrazine is known to enter the

chloroplasts, the mutagenic efficiencies of the chloroplast fraction and the supernatant were also investigated.

MATERIAL AND METHODS

Schizosaccharomyces pombe Lindner (strain *ade7-C8* from Professor Munz, Bern, Switzerland) was used as a test organism. It is a homothallic strain (*h90*) which gives rather high spontaneous reversion rates.

Erlenmeyer flasks containing 100 ml of yeast extract in liquid medium (5 g yeast extract, 30 g dextrose/l) were inoculated with the strain and agitated (for 2 days) for aeration until the stationary phase of growth was reached (about 0.7×10^8 cells/ml). For each treatment, 5 ml of this suspension was centrifuged (Sorval, 10 min, $2500 \times g$) and washed twice with sterile water for removal of the complete medium. Water was replaced by 5 ml atrazine solutions at different concentrations (pure atrazine or commercial formulation with and without activation by maize, see below).

The cells were resuspended and agitated (1 hr) in different atrazine solutions. After mutagenic treatment, the suspension was centrifuged, washed three times and resuspended in an equal volume of sterile distilled water: 0.2 ml of untreated or treated cell suspensions were spread without dilution on the surface of minimal medium (6.7 g nitrogen base, 10 g dextrose, 13 g agar/l). The plates were incubated (30°C) and scored for revertants 7 days after treatment. To determine cell survival, suspensions were diluted to 1×10^5 cells/ml and 0.2 ml of the diluted samples was spread on complete medium (5 g yeast extract, 30 g dextrose, 11 g agar/l). The plates were incubated (at 30°C) and counted after 5 days. Revertant colonies were white on minimal medium whereas colonies of the strain *ade7-C8* were red on complete medium.

For each of the figures mutation frequency has been corrected for survival levels, by calculating the ratio

$$M(x) = Nm(x)/Ns(x)$$

where $M(x)$ is the induced mutation frequency, $Nm(x)$ the yield of induced mutants and $Ns(x)$ the surviving fraction of cells.[6] The survival curves were previously shown to be linear.[11]

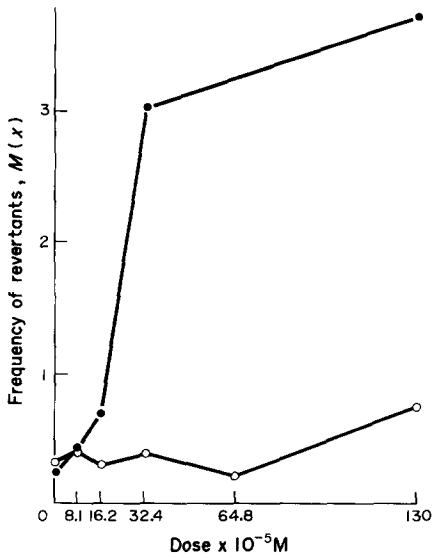

FIG. 1. Comparison of the effects of pure atrazine with (●— ●) and without (○ -- ○) activation by maize S1 fraction. $M(x)$: Mutation frequency corrected for survival.

The water solubility of the pure compound and also to a lesser extent, the commercial formulation, limited the range of concentrations that could be tested. Maize chloroplast fraction was extracted following ARNON's technique.[2]

RESULTS

In Fig. 1, the mutagenic effects of pure atrazine are compared in experiments with or without activation by the S1 fraction. It can be seen that the response obtained after activation with maize S1 fraction has a quadratic component whereas without activation, the response is linear.

In Fig. 2, the mutagenic effects of the commercial formulation of atrazine are compared with and without activation; the curve obtained after activation also has a quadratic component.

In Fig. 3, the effect of the chloroplast fraction is compared with that of the supernatant. The fact that the mutagenic activity is higher in Fig. 3 than in Fig. 1 strongly suggests a concentrating of the product mutagen in the chloroplast fraction.

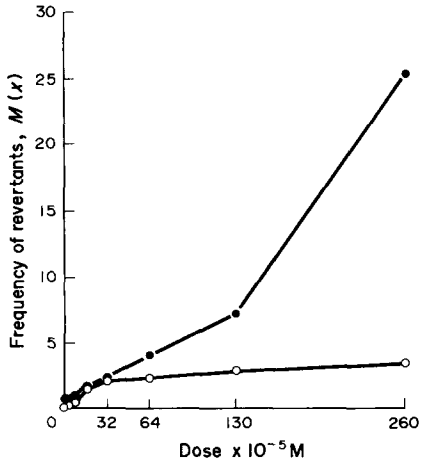

FIG. 2. Comparison of the effects of commercial atrazine with (● — ●) and without (○ — ○) activation by maize S1 fraction. $M(x)$: Mutation frequency corrected for survival.

DISCUSSION AND CONCLUSIONS

In the literature, there are discrepancies about the mutagenic effects of atrazine. This may be due to the commercial formulations used for mutagenicity testing. The 99% pure compound has been tested only in a few experiments.[3,11] In fact, the commercial formulations can differ in many respects:

(i) variation in the concentration of active substances (as herbicide) which include not only pure atrazine, but also other triazines such as simazine or promazine;

(ii) variation in the physical state of the active substances: hygroscopic powder or liquid;

(iii) occurrence of added solvent(s) and possible impurities, all possibly being capable of modifying the effects of triazines or being mutagenic themselves.

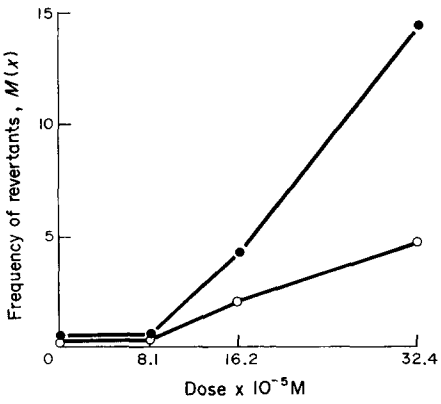

FIG. 3. Comparison of the effects of the chloroplast (● — ●) fraction and the supernatant (○ — ○) of maize treated with pure atrazine. $M(x)$: Mutation frequency corrected for survival.

In some experiments, the herbicide was used in saturated solutions[23,24] or as sprays.[5] These experimental conditions are difficult to reproduce. Another variable is the sensitivity of the plant used for testing. The species previously tested for clastogenicity are *Hordeum*,[22,23] *Vicia*[24] and *Sorghum*,[6–9] all known to be more sensitive species than *Nigella*. Metabolic pathways of the herbicides have been shown to be different in sensitive and resistant species.[5]

The metabolic pathway of atrazine is well documented.[16] It is partly detoxified in the cytoplasm of the target tissues before entering chloroplasts in which it accumulates until it reaches an equilibrium.[19] The uptake of the herbicide by chloroplasts or their metabolic product(s) is an important step for induction of plant lethality since it is a primary site of action for explaining some differences in sensitivity among some plant species.

The present experiments confirm the possibility of activating the herbicide atrazine with maize tissues *in vivo*. The effects obtained with the S1 fraction in *Schizosaccharomyces pombe* are comparable to those obtained in *Saccharomyces cerevisiae*.[13] However, the nature of the mutagenic transformation in plants is still unknown. More recently, microsomal fractions of plants (S9) were tested and *in vitro* activation of atrazine and other related compounds into product mutagens was observed.[4]

Since the *in vivo* transformation of the commercial formulation is far more important than the transformation of the pure compound, the main point to investigate in the future is whether substances other than triazines are involved, either as promutagens or as direct mutagens. Some of our current experiments are investigating this line of research.

REFERENCES

1. Andsersen K. J., Leighty E. G. and Takahashi M. T. (1972) Evaluation of herbicides for possible mutagenic properties. *J. Agric. Food Chem.* **20,** 649–656.
2. Arnon D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Pl. Physiol.* **24,** 1–15.
3. Chollet M. C., Degraeve N., Gilot-Delhalle J., Colizzi A., Moutschen J. and Moutschen-Dahmen M. (1982) Mutagenic efficiency of atrazine with and without metabolic activation. *Mutat. Res.* **97,** 237–238.
4. Gentile J. M., Gentile G. J. and Plewa M. J. (1986) *In vitro* activation of chemicals by plants: a comparison of techniques. *Mutat. Res.* **164,** 53–58.
5. Hamilton R. H. (1964) Tolerance of several grass species to 2-chloro-*s*-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *J. Agric. Food Chem.* **12,** 14–17.
6. Lee K. C., Rao G. M., Barnett F. L. and Liang G. H. (1974) Further evidence of meiotic instability induced by atrazine in grain *Sorghum*. *Cytologia* **39,** 697–702.
7. Liang G. H. L., Feltner, K. C., Liang Y. T. S. and Morrill J. L. (1967) Cytogenetic effects and response of agronomic characters in grain *Sorghum* (*Sorghum vulgare* Pers.) following atrazine application. *Crop Sci.* **7,** 245–248.
8. Liang G. H. L., Feltner K. C. and Russ O. G. (1969) Meiotic and morphological response of grain sorghum to atrazine, 2,4-D, oil, and their combinations. *Weed Sci.* **17,** 8–12.
9. Liang G. H. L. and Liang Y. T. S. (1972) Effects of atrazine on chromosomal behavior in *Sorghum*. *Can. J. Genet. Cytol.* **14,** 423–427.
10. Loprieno N., Barale R., Mariani L., Presciuttini S., Rossi A. M., Sbrana I., Zaccaro L., Abbondandolo A. and Bonatti S. (1980) Results of mutagenicity tests on the herbicide atrazine. *Mutat. Res.* **74,** 250.
11. Mathias M. (1987) Comparaison de la génotoxicité de deux préparations d'atrazine chez la levure *Schizosaccharomyces pombe* Lindner et chez la plante *Nigella damascena* L. *Bull. Soc. R. Sci. Liège* **5–6,** 425–432.
12. Müller A., Ebert E. and Gast A. (1972) Cytogenetic studies with atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) on plants. *Experientia* **28,** 704–705.
13. Plewa M. J. and Gentile J. M. (1975) A maize-microbe bioassay for the detection of proximal mutagenicity of agricultural chemicals. *Maize Genet. Coop. News Lett.* **49,** 40–43.
14. Plewa M. J. and Gentile J. M. (1976a) Mutagenicity of atrazine: a maize–microbe bioassay. *Mutat. Res.* **38,** 287–292.
15. Plewa M. J. and Gentile J. M. (1976b) Plant activation of herbicides into environmental mutagens: the waxy reversion bioassay. *Maize Genet. Coop. News Lett.* **50,** 44.
16. Plewa M. J. and Gentile J. M. (1982) The activation of chemicals into mutagens by green plants. Pages 401–420 *in* A. Hollaender and F. J. De Serres, eds *Chemical mutagens, principles and methods for their detection*, Vol. 7. Plenum Press, New York.
17. Plewa M. J., Wagner E. D. and Gentile J. M. (1979) Analysis of the mutagenic properties of pesticides incorporating animal and plant activation. *Envir. Mutagen.* **1,** 142.
18. Plewa M. J., Wagner E. D., Gentile G. J. and Gentile J. M. (1984) An evaluation of the genotoxic properties of herbicides following plant and animal activation. *Mutat. Res.* **136,** 233–245.
19. Suimabukuro R. H. and Swanson H. R. (1969) Atrazine metabolism, selectivity and mode of action. *J. Agric. Food Chem.* **17,** 199–206.
20. Shirasu Y., Moriya M., Kato K., Furuhashi A. and Kada T. (1976) Mutagenicity screening of pesticides in the microbial system. *Mutat. Res.* **40,** 19–30.
21. Siebert D. and Lemperle E. (1974) Genetic effects of herbicides: induction of mitotic gene conversion in *Saccharomyces cerevisiae*. *Mutat. Res.* **22,** 111–120.
22. Stroev V. S. (1970) Cytogenetic activity of the herbicides atrazine, chloro-IPC, and paraquate. *Genetika* **6,** 31–37.
23. Wuu K. D. and Grant W. F. (1966) Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). *Can. J. Genet. Cytol.* **8,** 481–501.
24. Wuu K. D. and Grant W. F. (1967) Chromosomal aberrations induced in somatic cells of *Vicia faba* by pesticides. *Nucleus* **10,** 37–46.