Mutation Research, 177 (1987) 247–254
Elsevier

MTR 04300

Patterns of epoxide metabolism by epoxide hydrolase and glutathione *S*-transferase associated with age and genotype in *Drosophila melanogaster*

J.A. Ottea [a], L.G. Harshman [a,b] and B. Hammock [a,c]

Departments of [a] Entomology, [b] Genetics and [c] Environmental Toxicology, University of California, Davis, CA 95616 (U.S.A.)

(Received 10 May 1986)
(Revision received 9 September 1986)
(Accepted 16 September 1986)

Keywords: Epoxide hydrolase; Glutathione *S*-transferase; Insect epoxide metabolism; Stilbene oxide; (*Drosophila melanogaster*); Genetic variation; Isozymes.

Summary

Epoxide hydrolase and glutathione *S*-transferase activities toward *trans*- and *cis*-stilbene oxides were measured in 3 strains of *Drosophila melanogaster*. Differences in age dependence, substrate selectivity and subcellular location were detected suggesting the presence of multiple forms of these enzymes. In addition, interstrain differences indicate the presence of genetic variation for epoxide hydrolase and glutathione transferase activities. These results illustrate a potential use of these assays in *D. melanogaster* to complement existing tests (e.g. recessive lethal tests or Ames assays) for evaluating the relationship between epoxide hydrolase and glutathione *S*-transferase levels and the genotoxicity of epoxides.

Advances in modern technology have led to the introduction of vast quantities of lipophilic chemicals into the environment. Epoxide-containing xenobiotics are one such group of public concern. These compounds contain highly strained cyclic ethers that are ubiquitous in our environment as industrial pollutants and naturally occurring dietary components (see Manson, 1980, for review). Although epoxides exhibit a wide range of stabilities (and therefore, reactivities), certain epoxides (e.g. aflatoxin B_1 epoxide) are some of the most potent mutagens known (Miller and Miller, 1977), a property thought to be due to the electrophilic nature of the epoxide ring (Miller, 1970; Tamura et al., 1982).

Correspondence: Dr. Bruce Hammock, Department of Entomology, University of California, Davis, CA 95616 (U.S.A.).

The ability to detect accurately the genotoxic potential of chemicals has not kept pace with their discovery and manufacture. The use of insects to test genotoxins offers a number of advantages over the currently used mammalian and bacterial models. Insects represent a level of evolutionary complexity between that of prokaryotes and mammals. Thus, insects may reflect the intricacies of mammalian systems more accurately than microbial systems currently in use. In addition, insects are cheaper to rear than mammals so they can be used in large numbers, and the short generation times of insects greatly reduce the time necessary to perform tests.

Since the pioneering work demonstrating the mutagenicity of allyl *iso*thiocyanate (Auerbach and Robson, 1944), the X-chromosome recessive lethal test and other assays based on *Drosophila melanogaster* have been used to test the mutagen-

0027-5107/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

icity of a variety of chemical classes (Vogel and Sobels, 1976; Vogel et al., 1980). It has been demonstrated that a good correlation (79%) exists between the in vivo carcinogenicity of the test chemical observed in mammals and mutagenicity as measured by the recessive lethal test (Vogel et al., 1980). This correlation could be improved by considering the role of biotransformation in the generation and inactivation of mutagens.

The metabolism of epoxides plays a critical role in their genotoxicity. Epoxides may be formed from the corresponding olefinic or aromatic compounds by the mixed-function oxidases (MFOs). These enzymes have received considerable attention for their role in the activation of indirect carcinogens (Grover et al., 1971; Vogel et al., 1980; Hallstrom et al., 1981). Without subsequent detoxication, these reactive intermediates have the potential to bind important biological macromolecules and disrupt regulatory processes. Thus, the enzymatic inactivation of epoxides is a process equally as important to consider for the accurate evaluation of genotoxic potential, and it has not been well studied.

There are two major pathways for the removal of epoxides in vivo: the conjugation of the epoxide with glutathione (GSH) catalyzed by glutathione *S*-transferase (GST, EC 2.5.1.18), and the addition of water across the epoxide ring by epoxide hydrolase (EH, EC 3.3.2.3). Both reactions facilitate elimination of the parent epoxide by the formation of metabolites that are more hydrophilic, although in some cases the metabolite may be more toxic (Sims et al., 1974; Elfarra et al., 1986).

The present report provides a basis for the further development of insect models for screening epoxide-containing mutagens. Data are reported that confirm the existence of an epoxide-metabolizing system in *Drosophila melanogaster* with some characteristics in common with mammalian EH and GST. In addition, among-strain diversity is demonstrated for the expression of these enzyme activities. This variability may be genetically based and will be useful in the development of test strains to evaluate the role of these enzymes in the prevention of epoxide-mediated mutagenicity.

Materials and methods

Chemicals

n-Hexanol (98%) and *iso*-octane (2,2,4-trimethylpentane) were purchased from Aldrich Chemical Company (Milwaukee, WI). Radiolabeled and unlabeled *cis*- and *trans*-stilbene oxides were prepared as previously described (Hammock and Hasegawa, 1983; Gill et al., 1983). Bovine serum albumin (fraction V) and glutathione (reduced) were purchased from Sigma Chemical Company (St. Louis, MO). Bio-Rad protein reagent was purchased from Bio-Rad Laboratories (Richmond, CA) and ACS-scintillation cocktail was purchased from Amersham (Arlington Heights, IL).

Drosophila strains

The stocks used in this study were Oregon R-C, Hikone-R and IIKA15, a strain constructed to contain a homozygous second chromosome isolated from a field population substituted into a Hochi-R background (Laurie-Ahlberg et al., 1980). Flies were reared in 0.4-pint bottles at 25°C with a 12:12 h light:dark cycle on a medium consisting of: cornmeal (6.2%), semolina (3.1%), sucrose (3.6%), dextrose (7.1%), agar (1.1%) and dead yeast (1.5%). Propionic acid (0.5%) was used as a preservative and live yeast was added to the medium surface. Bottles of Hikone-R and IIKA15 were initiated with 50 males and 50 females in accordance with Laurie-Ahlberg et al. (1980) while Oregon R-C bottles were started with only 20 females and 20 males following the convention employed in this laboratory. The adults were cleared from the bottles after 48 h. The possible effect of the resulting difference in larval densities on the enzyme activities measured is under investigation. Male or virgin female progeny were collected under CO_2 anesthesia and held 100/bottle at 25°C on medium without added live yeast. For the age dependence study, adults were transferred to fresh bottles every second day to avoid the effects of microbial build-up on the medium surface. Immediately before the flies were assayed, they were transferred to an empty bottle and immobilized by introducing a gentle flow of CO_2 for approximately 5 min. They were then counted, weighed and transferred to a Ten Broeck all-glass homogenizer on ice.

Preparation of enzymes

Experimental groups consisted of 100–1000 virgin female of male flies anesthetized with CO_2 and homogenized in 0.25 M sucrose with 30 strokes of a Ten Broeck all-glass homogenizer (final concentrations ca. 100 flies/ml). Unless otherwise indicated, this homogenate was centrifuged for 10 min at $10\,000 \times g$, the pellet was discarded, and the supernatant was subjected to further centrifugation at $100\,000 \times g$ for 60 min. The pellet from the spin (microsomes) was resuspended in 1.5 ml of 0.25 M sucrose; the supernatant (cytosol) was used as such. For optimization of enzyme assays, fractions were stored at −70°C in 0.5-ml aliquots until assayed. For all other tests fresh homogenates were used. To determine the effects of freeze/thaw on activities, enzyme aliquots were assayed as fresh homogenates and after freezing.

Enzyme assays

Epoxide hydrolase and glutathione transferase were measured radiometrically by the procedure of Gill et al. (1983) using *trans*- and *cis*-stilbene oxides as substrates. A typical reaction mixture contained enzyme (microsomal or cytosolic fractions, 0–100 μg protein) in 200 μl buffer (sodium phosphate, pH 6–8 or ammonia-ammonium chloride, pH 9 and 10). For GST assays, reduced GSH (5 mM final concentration, freshly prepared in buffer) was added. Reactions were initiated by the addition of 2 μl of substrate (5×10^{-3} M initial concentration in ethanol) and terminated following varying incubation times (0–80 min) by the addition of equal volumes of solvent (*iso*-octane for EH or *n*-hexanol for GST assays) and immediate, vigorous vortexing. Following centrifugation to break emulsions ($1000 \times g$ for 3 min), a 150-μl aliquot of the aqueous phase was transferred to aqueous scintillation cocktail for scintillation counting. All values were corrected for non-enzymatic hydration, and linearity of product formation with time and protein was ensured. Units are defined as pmoles diol (EH) or conjugate (GST) formed. Protein concentrations were estimated by the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis

Mixed model, multiway analysis of variance (ANOVA) was performed using the General Linear Models Program from a SAS package. Tukey's test was used for multiple comparisons. For all statistical test, the critical level was set at 0.05.

Results

Epoxide hydrolase and glutathione transferase activities measured were linear within the ranges of protein (0–100 μg/assay) and incubation times (0–80 min) tested. Only data from incubations resulting in less than 30% metabolism are reported in this study. Smooth, bell-shaped pH/activity plots with optima at pH 8.0 were measured for all activities (data not shown). Three cycles of freezing then thawing had no major adverse effect on patterns of activities measured in optimization experiments.

EH and GST activities were measured in the two subcellular fractions and the $10\,000 \times g$ supernatant (Table 1). The major EH activity was observed in the microsomes while GST activity was predominantly cytosolic. Supernatants of $10\,000 \times g$ centrifugation were tested to determine whether further ($100\,000 \times g$) centrifugation was necessary for optimal measurement of these enzyme activities. Some activities in this crude supernatant (e.g. EH with CSO and GST with TSO) did not reflect the levels measured in the post-microsomal fractions in that more activity was detected in the separated fractions than would be predicted based on analysis of the $10\,000 \times g$ supernatant. Therefore, this additional step in sample preparation was employed for subsequent determinations of activities.

Major differences in the rate of metabolism of the two substrates were measured for both EH and GST activities in Oregon R-C fruit flies (Table 1). For the microsomal EH, hydrolysis of CSO was nearly 7-fold higher than that with TSO. In contrast, the cytosolic activities were equivalent for the two substrates. In tests for GST activity, no conjugation was detected with TSO using microsomes as the enzyme source while low but significant levels of activity were measured with CSO. Cytosolic GST activity was 2-fold higher with TSO than CSO as substrate. Because GST/TSO activity is abundant in the cytosol, the absence of the corresponding microsomal activity

TABLE 1

SUBCELLULAR DISTRIBUTION OF EH AND GST ACTIVITIES IN OREGON R-C FRUIT FLIES [a]

	pmoles/min/mg protein		pmoles/min/fly equiv.	
	TSO	CSO	TSO	CSO
Epoxide hydrolase				
10000× *g* supernatant	115 ± 6	230 ±37	3.8±0.4	8.0±0.9
Microsomes	83.1± 5.7	572 ±52	1.5±0.2	9.7±1.0
Cytosol	59.7± 6.6	59.2± 7.0	2.0±0.1	2.4±0.6
Glutathione S-transferase				
10000× *g* supernatant	149 ±60	207 ±41	5.8±2.2	7.9±1.1
Microsomes	ND [b]	51.5±15.5	ND [c]	0.9±0.2
Cytosol	334 ±75	167 ±53	10.9±1.8	5.7±1.3

[a] 4-day-old, virgin female flies were used as enzyme source and *trans-* and *cis*-stilbene oxides (TSO and CSO, respectively) were used as substrates. Data represent mean activity±S.E. based on triplicate assays of 4–6 different homogenates, each from different generation of flies.

[b] Not detectable under assay conditions where 10 units of activity were detected as above background.

[c] Not detectable under assay conditions where 0.5 units of activity were detected as above background.

indicated that the microsomal preparations were relatively free of cytosolic contamination.

Differences in EH activity toward the two substrates were measured also with respect to the age of the flies used as enzyme source (Figs. 1 and 2). As can be seen from the inserts, protein levels did not remain constant at the ages tested. Thus, all activities were expressed relative to the number of flies used per assay (fly equivalents). In microsomal fractions TSO hydrolysis remained rela-

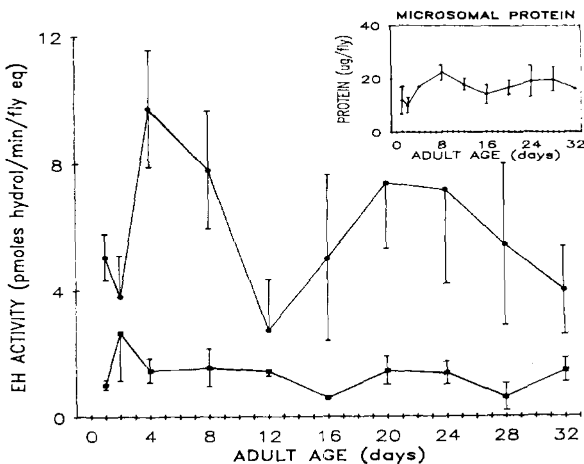

Fig. 1. Age dependence of microsomal epoxide hydrolase activities (TSO, squares; CSO, circles) of Oregon R-C homogenates. Points represent mean activity (pmoles substrate hydrolyzed/min/fly equivalent)±95% confidence interval (CI) at 30°C based on triplicate assays of 5 homogenates (150–200 virgin female flies/homogenate) with each assay run on different days. Absence of bars indicated that CI is within the data point. Insert shows the age dependence of microsomal protein levels±CI expressed on a per fly basis.

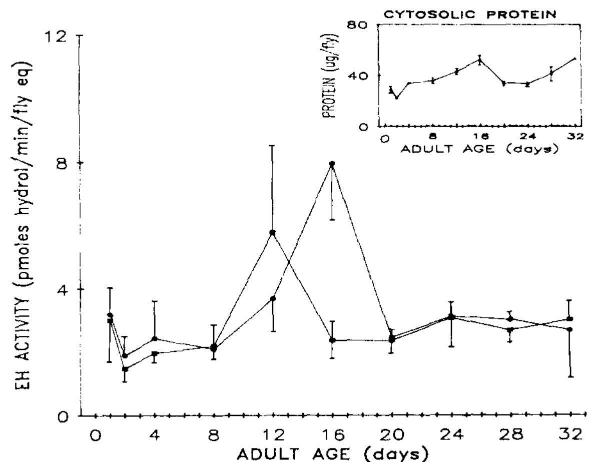

Fig. 2. Age dependence of cytosolic epoxide hydrolase activities (TSO, squares; CSO, circles) of Oregon R-C homogenates. Points represent mean activity (pmoles substrate hydrolyzed/min/fly equivalent±95% CI) at 30°C based on triplicate assays of 5 homogenates (150–200 virgin female flies/homogenate) with each assay run on different days. Absence of bars indicated that CI is within the data point. Insert shows the age dependence of cytosolic protein levels±CI expressed on a per fly basis.

tively constant at the times tested (Fig. 1). Activity with CSO in this fraction peaked at day 4, dropped sharply at day 12 and then increased to a broad peak between days 20 and 24. Similar patterns of activity were observed in tests with each of the 5 homogenates used providing additional evidence that this decrease in microsomal hydration of CSO actually occurs.

Patterns of EH activity in the cytosol were distinct compared to the microsomal activities, and with respect to the substrate used (Fig. 2). In contrast to patterns measured in the microsomes, the activity profiles for the two substrates were similar during the early and late ages studied. However, peak activities were different and were measured at days 12 and 16 for TSO and CSO hydrolysis, respectively. Patterns of protein content in this fraction were also different from those measured in the microsomes. Differences in age dependence were also measured for GST activities (Fig. 3). As with EH, age profiles were distinctive with respect to both subcellular location and substrate used.

The age dependences of these activities were measured in male flies as well. No qualitative differences were seen in the patterns of activities. However, levels of activity in males (whether expressed as per fly or per mg protein) were 20–30% less than those measured with females (data not shown).

Variable levels of EH and GST activities were measured in the 3 strains of *D. melanogaster* tested (Table 2). ANOVA tests revealed significant overall heterogeneity among strains for EH and GST ($P < 0.05$ and 0.01, respectively). For EH, both the Hikone-R and IIKA15 strains had cytosolic activities significantly higher than those in the Oregon R-C strain ($P < 0.01$ and 0.05 for TSO and CSO, respectively). No statistically significant differences in EH were measured in microsomal fractions. Similarly, GST activities were most variable in cytosol. In addition, the ranking of activities in the 3 strains differed depending on the substrate used. In tests with TSO, Hikone-R activity was significantly higher than the other two strains ($P < 0.05$) while with CSO, both Hikone-R and IIKA15 activities were higher than Oregon R-C.

Qualitative differences in EH and GST activities were also measured in these strains. Statistical analysis indicated overall substrate selectively for

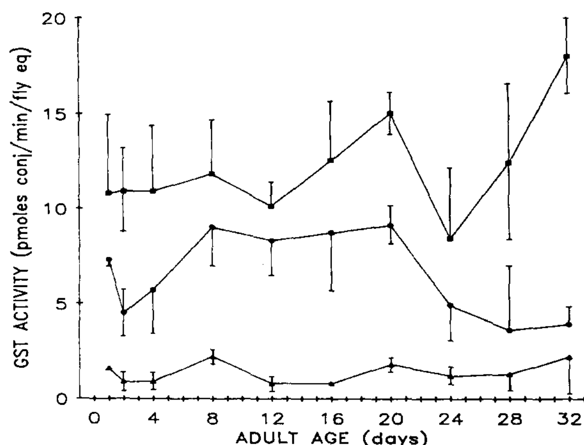

Fig. 3. Age dependence of glutathione *S*-transferase activities from microsomes (CSO, triangles; TSO not detected) and cytosol (TSO, squares; CSO, circles) of Oregon R-C homogenates. Points represent mean activities (pmoles substrate conjugated/min/fly equivalent ± 95% CI) at 30°C based on triplicate assays of 5 homogenates (150–200 female flies/homogenate) with assays run on different days. Absence of bars indicates that CI is within the data point.

TABLE 2

EPOXIDE HYDROLASE AND GSH TRANSFERASE ACTIVITIES [a] IN 3 STRAINS OF *D. melanogaster*

	Microsomes		Cytosol	
	TSO	CSO	TSO	CSO
Epoxide hydrolase				
Oregon R-C	2.6±0.8	3.8±0.7	1.5±0.2	1.9±0.4
IIKA15	1.0±0.1	3.5±0.2	4.9±1.1	5.3±1.6
Hikone-R	1.4±0.4	3.7±0.5	5.2±0.6	4.0±0.2
GSH transferase				
Oregon R-C	ND [c]	0.9±0.2	10.9±1.1	4.5±0.7
IIKA15	ND	ND	7.1±1.7	7.2±0.8
Hikone-R	ND	0.9±0.1	16.8±3.1	10.7±3.2

[a] 2-day-old, virgin female flies were used as enzyme source and *trans*- and *cis*-stilbene oxides (TSO and CSO, respectively) were used as substrates. Data represent mean activity (pmoles substrate metabolized/min/fly equivalent) ± S.E. based on triplicate assays of 3–6 different homogenates, each from a different generation of flies.

[b] Bars denote activities that are not significantly different based on ANOVA tests at $P < 0.05$.

[c] Not detectable under assay conditions where 0.5 units of activity were detected as above background.

both EH and GST ($P < 0.05$ and 0.01, respectively). However, this selectivity was more evident in some strains than others (e.g. cytosolic GST activity in Oregon R-C and IIKA15). In addition, the effect of the subcellular location on the enzyme activities varied among these strains. For example, EH/CSO activities were predominantly microsomal in the Oregon R-C strain but cytosolic in the other two strains.

Discussion

Drosophila melanogaster has been shown previously to be valuable for predicting the mammalian genotoxicity of compounds from a number of chemical classes including those which required metabolic activation (Vogel and Sobels, 1976; Nix et al., 1981; Hallstrom et al., 1981). However, the effects of detoxication of these reactive intermediates on their genotoxicity have not been well characterized (see Baars, 1980, for review). Epoxides are of special interest for study because, in some cases, they arise via the oxidation of olefins but also exist in nature as potentially toxic contaminants of our food.

Epoxide hydrolase and glutathione *S*-transferase activities were measured from both microsomal and cytosolic fractions of *Drosophila melanogaster*. Previously, Baars et al. (1979) reported the hydrolysis of another epoxide substrate, styrene oxide, by EH in the microsomes of *D. melanogaster*. In a more recent study, Jansen et al. (1986) measured EH activity with styrene oxide in the cytosol of this insect. These activities with this substrate were more than 4-fold greater than the highest rates measured here using TSO or CSO which may reflect steric hindrance of the epoxide ring in the latter compounds.

Differences in subcellular location, substrate selectivity and age profiles for EH activity suggest the presence of multiple forms of this enzyme in *D. melanogaster*. EH activity with CSO in the microsomes was greater than with TSO, while no major differences were measured between the two substrates in the cytosol. This pattern of activity was qualitatively similar to that seen in humans and rhesus monkeys (Silva and Hammock, 1986), other mammals (Gill et al., 1983) and *Trichoplusia ni*, a phylogenetically distinct insect (Ottea and Hammock, 1986). In addition, the age profiles of EH activity measured in this study were distinct for the subcellular location and substrate used for assay. Whether these differences in patterns, or the fluctuations in microsomal activity with CSO are correlated with the intrinsic biological role of EH in this insect is presently under investigation.

Glutathione *S*-transferase activitity was also detected in both subcellular fractions of *Drosophila melanogaster*. These results were in contrast to earlier studies where GST activity was not detected using the epoxides styrene 7,8-oxide (Baars et al., 1979; Jansen et al., 1984) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (Jansen et al., 1984) in the cytosol of Hikone-R homogenates. It is possible that this discrepancy indicates selectivity of these enzymes for the type of substrates used in the present study. It is also feasible that the high reactivity of styrene oxide and 1,2-epoxy-3-(*p*-nitrophenoxy)-propane precluded the detection of low levels of GST activity. The lower reactivities and the lack of health hazards associated with TSO and CSO make them valuable model substrates for the evaluation of epoxide-metabolizing systems. However, the relative strengths and weaknesses of all the available assays should be considered when selecting analytical systems (Wixtrom and Hammock, 1985) and no generalizations should be made until the enzymes involved have been carefully characterized with a number of different substrates.

As in tests with EH, differences in age profiles, subcellular distribution, and substrate selectivity support a hypothesis for the presence of multiple forms of GST in this insect. Different patterns for the 3 GST activities with respect to adult age. Microsomal GST activity was detectable with CSO only, while cytosolic enzymes were active toward both TSO and CSO. The fact that a microsomal GST activity toward CSO has recently been measured from mouse liver (Moody et al., 1985) reflects another similarity between the epoxide-metabolizing systems in mammals and *D. melanogaster*. In general, the age profiles for both GST and EH were unlike those found for a number of mixed-function oxidase (MFO) activities from *D. melanogaster* (Waters et al. 1984), houseflies (Perry and Buckner, 1970) and the cockroach, *Diploptera punctata* (Feyereisen and Farnsworth, 1985).

Varying levels of EH and GST activities, and differing degrees of substrate selectivity seen in the strains tested suggest a genetic basis for this diversity. Similar results were reported for MFO activities in *D. melanogaster* (Vogel, 1980; Hallstrom et al., 1984; Waters et al., 1984). In contrast to the results reported here, Baars et al. (1979) found no differences in EH or GST activities using styrene oxide and 1-chloro-2,4-dinitrobenzene as substrates in tests with 3 strains (Hikone-R, Berlin-K and a white-eyed strain). At present it is not known whether these data reflect genotypic differences among the strains used in these and the previous tests, or actual differences in the substrate selectivity of these enzymes.

The findings of our research illustrate that epoxide metabolism in *Drosophila melanogaster* satisfies 3 criteria for use as a model for the disposition of mutagens in humans. First, epoxide hydrolase and glutathione *S*-transferase activities were detectable in subcellular fractions of adults. Second, the activities measured from these flies had some characteristics in common with the corresponding human enzymes. Finally, the diversity of these activities may have a genetic basis. Greater variability may be found as the number of strains examined is increased. This diversity will be useful in finding a strain of *D. melanogaster* with a system for the metabolism of epoxides that is similar to that in humans. In addition, tests with strains possessing different levels of these enzymes will provide basic information regarding the role of these enzyme activities in the metabolism of potentially toxic epoxides.

Acknowledgments

This work was supported in part by NIEHS Grant 5 ROI ES02710-05 and NSF DCB-85 18697. J.A. Ottea was supported in part by a Jastro-Shields Research Scholarship. Lawrence Harshman was supported in part by NIH GM22221 and B.D. Hammock and supported by NIEHS Research Career Development Award 5 KO4 ES500107-5.

We thank C.P. McHugh and S.S. Duffey (Dept. of Entomology, U.C. Davis) for statistical and editorial assistance, respectively, and Tim Prout (Dept. of Genetics, U.C. Davis) for his encouragement. We also thank Cathy Laurie-Ahlberg for the chromosome substitution lines and Bruce Cochrane for helpful discussions.

References

Auerbach, C., and J.M. Robson (1944) Production of mutations by allyl *iso*thiocyanate, Nature (London), 154, 81.

Baars, A.J. (1980) Biotransformation of xenobiotics in *Drosophila melanogaster* and its relevance for mutagenicity testing, Drug Metab. Rev., 11, 191–221.

Baars, A.J., M. Jansen and D.D. Breimer (1979) Xenobiotica-metabolizing enzymes in *Drosophila melanogaster*, Activities of epoxide hydratase and glutathione *S*-transferase compared with similar activities in rat liver, Mutation Res., 62, 279–291.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analyt. Biochem., 72, 248–254.

Elfarra, A.A., I. Jakobson and M.W. Anders (1986) Mechanism of *S*-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity, Biochem. Pharmacol., 35, 283–288.

Feyereisen, R., and D.E. Farnsworth (1985) Developmental changes of microsomal cytochrome P-450 monooxygenases in larval and adult *Diploptera punctata*, Insect Biochem., 15, 755–761.

Gill, S.S., K. Ota and B.D. Hammock (1983) Radiometric assays for mammalian epoxide hydrolases and glutathione *S*-transferase, Analyt. Biochem., 131, 273–282.

Grover, P.L., P. Sims, E. Huberman, H. Marquardt, T. Kuroki and C. Heidelberger (1971) in vitro transformation of rodent cells by K-region derivatives of polycyclic hydrocarbons, Proc. Natl. Acad. Sci. (U.S.A.), 68, 1098–1101.

Hällström, I., A. Sundvall, U. Rannug, R. Grafström and C. Ramel (1981) The metabolism of drugs and carcinogens in isolated subcellular fractions of *Drosophila melanogaster*, I. Activation of vinyl chloride, 2-aminoanthracene and benzo[*a*]pyrene as measured by mutagenic effects in *Salmonella typhimurium*, Chem.-Biol. Interact., 34, 129–143.

Hällström, I., A. Blanck and S. Atuma (1984) Genetic variation in cytochrome P-450 and xenobiotic metabolism in *Drosophila melanogaster*, Biochem. Pharmacol., 33, 13–20.

Jansen, M., A.J. Baars and D.D. Breimer (1984) Cytosolic glutathione *S*-transferases in *Drosophila melanogaster*, Biochem. Pharmacol., 33, 3655–3659.

Jansen, M., A.J. Baars and D.D. Breimer (1986) Microsomal and cytosolic epoxide hydrolase in *Drosophila melanogaster*, Biochem. Pharmacol., 35, 2229–2232.

Laurie-Ahlberg, C.C., G. Maroni, G.C. Bewley, J.C. Lucchesi and B.S. Weir (1980) Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*, Proc. Natl. Acad. Sci. (U.S.A.), 77, 1073–1077.

Manson, M.M. (1980) Epoxides — is there a human health problem? Br. J. Ind. Med., 37, 317–336.

Miller, J.A. (1970) Carcinogenesis by chemicals: an overview

— G.H.A. Clowes Memorial Lecture, Cancer Res., 30, 559–576.

Miller, J.A., and E.C. Miller (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Laboratory, New York, pp. 605–627.

Moody, D.E., D.N. Loury and B.D. Hammock (1985) Epoxide metabolism in the liver of mice treated with clofibrate (ethyl-α-(*p*-chlorophenoxyisobutyrate)), a peroxysome proliferator, Toxicol. Appl. Pharmacol., 78, 351–362.

Nix, C.E., B. Brewen and J.L. Epler (1981) Microsomal activation of selected polycyclic aromatic hydrocarbons and aromatic amines in *Drosophila melanogaster*, Mutation Res., 88, 291–299.

Ottea, J.A., and B.D. Hammock (1986) Optimization of assay conditions for epoxide metabolizing enzymes in *Trichoplusia ni*, Insect Biochem., 16, 319–325.

Perry, A.S., and A.J. Buckner (1970) Studies on microsomal cytochrome P-450 in resistant and susceptible houseflies, Life Sci., 9, 335–350.

Silva, M.H., and B.D. Hammock (1986) Affinity purification of cytosolic epoxide hydrolase from human, nonhuman primate, rabbit, and rodent liver, Arch. Biochem. Biophys. in press.

Sims, P., P.L. Grover, A. Swaisland, K. Pal and A. Hewer (1974) Metabolic activation of benzo[*a*]pyrene proceeds by a diol-epoxide, Nature (London), 252, 326–328.

Tamura, N., K. Takahashi, N. Shirai and Y. Kawazoe (1982) studies on chemical carcinogens, XXI. Quantitative structure–mutagenicity relationship among substituted styrene oxides, Chem. Pharm. Bull., 30, 1393–1400.

Vogel, E. (1980) Genetical relationship between resistance to insecticides and procarcinogens in two Drosophila populations, Arch. Toxicol., 43, 201–211.

Vogel, E., and F.H. Sobels (1976) The function of Drosophila in genetic toxicology testing, in: A. Hollaender (Ed.), Chemical Mutagens, Principles and Methods for their detection, Vol. 4, Plenum, New York, pp. 93–142.

Vogel, E., W.G.H. Blijleven, P.M. Klapwijk and J.A. Zijlstra (1980) Some current prospectives of the application of Drosophila in the evaluation of carcinogens, in: G.M. Wiliams et al. (Eds.), The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 125–147.

Waters, L.C., C.E. Nix, K.M. Solden and J.L. Epler (1984) Effects of genotype and age on mixed-function oxidase activities in adult *Drosophila melanogaster*, Mutation Res., 139, 51–55.

Wixtrom, R.N., and B.D. Hammock (1985) Membrane-bound and soluble-fraction epoxide hydrolases: methodological aspects, in: D. Zakim and D.A. Vessey (Eds.), Biochemical Pharmacology and Toxicology, Vol. 1, Methodological Aspects of Drug Metabolizing Enzymes, Wiley, New York, pp. 1–93.