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DIFFERENTIAL REGULATION OF HEPATIC GLUTATHIONE TRANSFERASE AND GLUTATHIONE PEROXIDASE ACTIVITIES IN THE RAT

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Abstract—The effects of the xenobiotics, i.e. butylated hydroxytoluene, β-naphthoflavone, isosafrole, pregnenolone-16α-carbonitrile, *trans*-stilbene oxide, 3-methylcholanthrene, phenobarbital, 3,3',4,4'-tetrachlorobiphenyl, 2,2',4,4',5,5'-hexachlorobiphenyl, on rat liver cytosolic glutathione transferase and glutathione peroxidase activities have been investigated. Although the glutathione transferase isozymes (measured by the specific substrates ethacrynic acid and Δ^5-androstene-3,17-dione) which have been shown to possess peroxidase activity were significantly increased, little or no increase in peroxidase activity (toward cumene hydroperoxide, *tert*-butyl hydroperoxide or hydrogen peroxide) was observed. Likewise during a 16-day time course following the administration of Aroclor 1254 or fireMaster BP-6 (each 500 mg/kg, i.p.), potent induction of glutathione transferase activities was seen without any significant increases in peroxidase activities. In fact during the second week of the time course, there were significant decreases in selenium-dependent glutathione peroxidase activity (toward hydrogen peroxide). The inverse regulation of these activities, i.e. the depression of selenium-dependent glutathione peroxidase activity following sustained induction of glutathione transferases, may have direct implications for the toxicity of the polyhalogenated aromatic hydrocarbons.

Glutathione peroxidase activity is present in most mammalian species and the tissues of these species as far as investigated [1–4]. It is represented on the one hand by a tetrameric selenium containing enzyme glutathione peroxidase (glutathione:hydrogen peroxide oxidoreductase, E.C. 1.11.1.9, Se-GSH-Px)‡ which is able to reduce hydrogen peroxide to water and organic hydroperoxides to the corresponding hydroxy compounds and on the other hand by certain isozymes of GST (E.C. 2.5.1.18), which catalyze the conjugation of GSH with electrophilic substrates. These isozymes possess a second catalytic function against organic hydroperoxides similar to Se-GSH-Px but cannot reduce hydrogen peroxide. Among different species as well as among the various tissues of one species considerably different ratios of Se-GSH Px and non-Se-GSH-Px activities have been observed [1–3]. In rat liver cytosol a part of the total GSH-Px activity against organic hydroperoxides and nearly the entire GSH-Px activity against hydrogen peroxide is due to the Se-GSH-Px. The GST isozymes from rat liver cytosol possessing most of the remaining GSH-Px activity against organic hydroperoxides are the three "B-type" transferases composed of the possible combinations of the subunits 1 (Ya; Mr 25,600) and 2 (Yc; Mr 28,000) [5, 6] corresponding to the isozymes GST 1-1, GST 1-2 and GST 2-2 in the new nomenclature of Jakoby *et al.* [7]. These subunits which possess high peroxidase activity can be distinguished from the subunits 3 (Yb; Mr 27,000) and 4 (Yb'; Mr 27,000) composing the "C-type" transferases (GST 3-3, GST 3-4 and GST 4-4) which possess only negligible peroxidase activity [5], by their substrate specificities. AD for example is turned over rapidly by isozymes containing the subunit 1(Ya), i.e. GST 1-1 and GST 1-2, while EA is a selective substrate for the subunit 2 (Yc), i.e. GST 1-2 and 2-2 isozymes. Recently Ketterer and co-workers observed a high GSH-Px activity against organic hydroperoxides of the purified homodimeric isozyme GST 5-5 (formerly GST E) [8].

In contrast to the Se-GSH-Px, for which no inducer has been found, GST can be induced by a wide range of xenobiotics. Potent inducers of cytosolic GST activities include the microsomal enzyme inducers, PB and MC as well as other polycyclic aromatic hydrocarbons [9–11], TSO [12–14], PCN, BNF and ISF [15], polyhalogenated aromatic hydrocarbons such as the polychlorinated biphenyls [16], 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [17] as well as phenolic antioxidants [18].

There are several hints that hepatic cytosolic GSH-Px and GST activities may be inversely related. Under conditions of acute selenium deficiency in the rat, where Se-GSH-Px was strongly depressed, the GST activity was enhanced [3]. When phenobarbital

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‡ *Abbreviations used:* GST, glutathione transferase; GSH-Px, glutathione peroxidase; Se-GSH-Px, selenium-dependent glutathione peroxidase; non-Se-GSH-Px, non-selenium-dependent glutathione peroxidase; GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; EA, ethacrynic acid; AD, Δ^5-androstene-3,17-dione; CHP, cumene hydroperoxide; *t*-BHP, *tert*-butyl hydroperoxide; BHT, 2,6-ditertiary-butyl-4-hydroxytoluene; BNF, β-naphthoflavone; ISO, isosafrole; PCN, pregnenolone-16α-carbonitrile; TSO, *trans*-stilbene oxide; MC, 3-methylcholanthrene; PB, phenobarbital; $(3,4)^2$Cl, 3,3'4,4'-tetrachlorobiphenyl; $(2,4,5)^2$Cl, 2,2',4,4',5,5'-hexachlorobiphenyl.

was administered to these Se-deficient rats, Se-GSH-Px was further depressed, while GST activities (toward CDNB and 1,2-dichloro-4-nitrobenzene) were further increased. Recently this inverse relationship between GSH-Px and GST activities seen during selenium deficiency was also observed in other organs, the kidney and duodenal mucosa [19]. Interestingly when these authors injected selenite, control levels of GST activity were restored within 48 hr. This inverse relationship has also been observed in the mouse, where mice placed on a selenium-deficient diet showed decreasing Se-GSH-Px activity, reaching undetectable levels after 17 days. At that time GST activity began to increase significantly [20]. Under conditions of selenium sufficiency, however, the situation is less clear. The administration of BHT, a potent inducer of GST activity (3×), led to no net increase in GSH-Px activity, although GST isozymes, which have been shown to possess peroxidase activity, were apparently induced [21]. In order to sort out the effects of inducers on the regulation of GSH-Px and GST activities, we investigated the effects of nine potent inducers on GST (measured with the substrates CDNB, EA and AD) and on GSH-Px activity (measured with hydrogen peroxide, CHP and *t*-BHP). In addition, a 16-day time course of these activities after administration of commercial polyhalogenated biphenyl mixtures is presented.

MATERIALS AND METHODS

Chemicals. CDNB (recrystallized from ethanol), EA, CHP, BHT, GSH (reduced form), and glutathione reductase (type III from yeast) were purchased from Sigma Chemical Co. (St. Louis, MO); *t*-BHP, TSO, BNF, and MC from Aldrich-Chemie (Steinheim, F.R.G.); ISO and PB from Merck-Schuchardt (Hohenbrunn/München, F.R.G.); NADPH from Boehringer Mannheim GmbH (F.R.G.); and hydrogen peroxide from Merck (Darmstadt, F.R.G.); PCN was generously provided by Upjohn Company (Kalamazoo, MI); Aroclor 1254 and fireMaster BP-6 were gifts from Bayer AG (Leverkusen, F.R.G.); and Michigan Chemical Co. (St. Louis, MI), respectively.

Synthesis. AD was synthesized according to [22]. The polychlorinated biphenyls $(3,4)^2$Cl and $(2,4,5)^2$Cl were synthesized from the corresponding halogenated benzidines via Sandmeyer reaction as modified by Roosmalen [23]. The compounds were purified by column chromatography (Aluminiumoxid 90, Art. 1097, Merck, Darmstadt, F.R.G.) and recrystallization from methanol. $(3,4)^2$Cl, m.p. 176–178°, lit. 173° [23] and $(2,4,5)^2$Cl, m.p. 102–103°, lit. 103–104° [24] were greater than 99% pure as determined by gas chromatography. Structural assignments were confirmed by proton magnetic resonance and mass spectroscopy.

Animal experiments. For the time course studies 6-week-old male Sprague–Dawley rats, 160–180 g from the Süddeutsche Versuchstierfarm, Tuttlingen, F.R.G., were fed a standard rat chow (Altromin 1324, Altromin GmbH & Co. KG, Lage, F.R.G.) and were housed in plastic cages on a fixed day and night cycle. The animals received a single i.p. injection of 500 mg/kg Aroclor 1254 or fireMaster BP-6 in corn oil (5 ml/kg). Groups of animals were killed 2, 4, 6, 8, 10, 13 and 16 days after treatment by cervical dislocation. All animals were fasted for 12 hr prior to killing.

For all other studies 8-week-old male Sprague–Dawley rats about 200–220 g from the Süddeutsche Versuchstierfarm were divided in groups of four animals, fed and housed as above. The animals were pretreated i.p. as follows: prior to killing TSO (400 mg/kg) and BHT (250 mg/kg) dissolved in corn oil were administered daily for 5 consecutive days, while PCN (50 mg/kg), MC (100 μmoles/kg), BNF (100 μmoles/kg), ISO (150 mg/kg) in corn oil and PB (400 μmoles/kg) in 0.9% saline were administered daily for three consecutive days. $(3,4)^2$Cl (150 μmoles/kg) and $(2,4,5)^2$Cl (150 μmoles/kg) in corn oil were administered on days 1 and 3 and the animals were killed by cervical dislocation on day 6. Corn oil treated animals (10 ml/kg) served as controls. Rats were fasted as above.

Preparation of liver cytosol. Each liver was perfused *in situ* with an ice-cold solution of 0.25 M sucrose containing 0.1 mM EDTA, pH 7.4. All further steps were carried out in this solution at +4°. The livers were excised, weighed and cut with scissors into small pieces, which were washed twice with the above mentioned solution and then homogenized with an Ultra Turrax (TP 18/10, Janke + Kunkel GmbH + Co. KG, Staufen, F.R.G.).

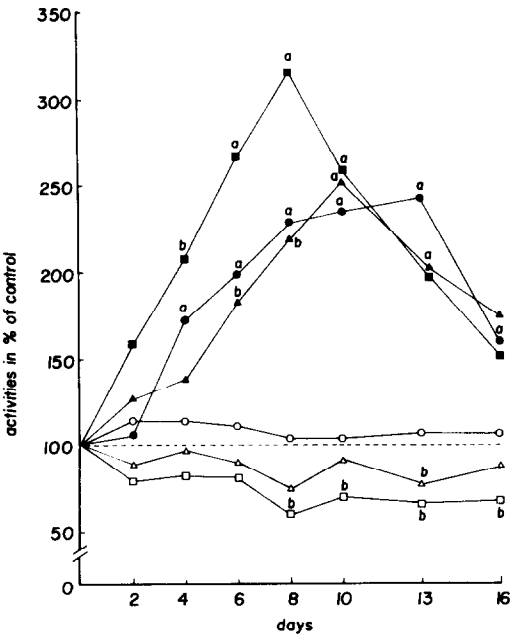
The homogenate was centrifuged in a Sorvall Centrifuge at 10,000 *g* for 20 min to remove the nuclear and mitochondrial fractions. The supernatant was centrifuged in a Beckmann ultracentrifuge for 1 hr at 100,000 *g*. The resulting supernatant was adjusted to a protein concentration of 12 or 14 mg/ml by the method of Lowry [25], frozen in liquid nitrogen and stored at −70° until used for the determination of enzyme activities.

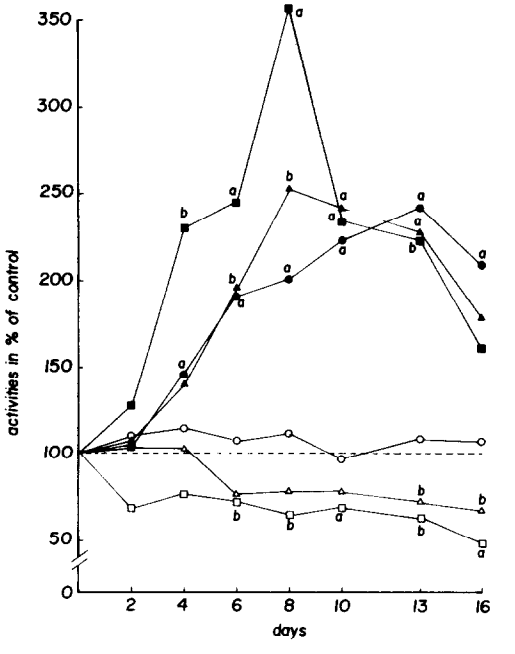
Enzyme assays. GSH-Px activity was determined by the coupled test procedure [26] using the reagents according to [27]. The reaction was started by addition of the peroxide and the linear decrease of NADPH absorption was recorded at 340 nm. GST activities with CDNB, EA [28] or AD [29] as substrates were determined as described. All enzyme assays were linear functions of protein concentration and of time and were carried out on a Shimadzu double beam spectrophotometer.

Statistics. Statistics were done by Dunnett's test for multiple comparisons with a control [30].

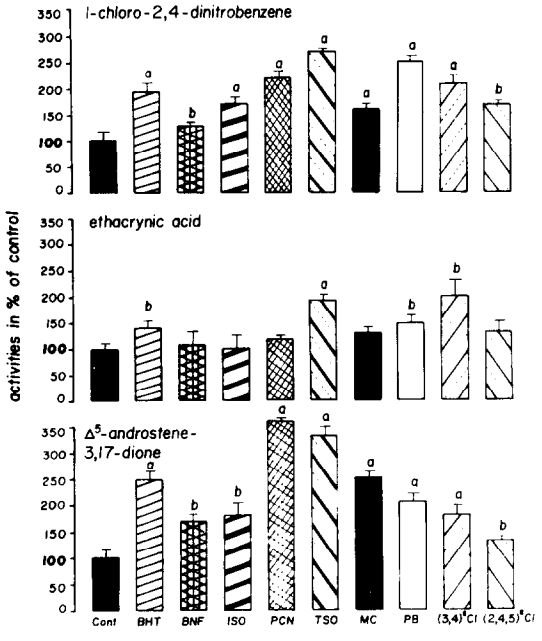
RESULTS

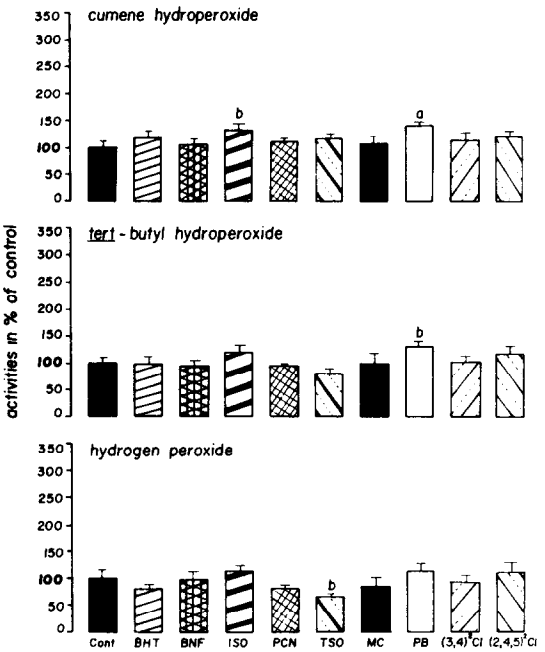
The time course of GST activities after administration of Aroclor 1254 (Fig. 1) with the broad-spectrum substrate CDNB and the selective substrates EA and AD showed significant increases with maxima at day 13 for CDNB activity (243% of control), day 10 for EA activity (252% of control) and at day 8 for AD activity (316% of control). At the same time total GSH-Px activity with CHP and *t*-BHP showed no significant changes; hydrogen peroxide activity, representing the Se-GSH-Px activity, showed a slight but significant decrease from day 8 to day 16. The time course of different activities after treatment with fireMaster BP-6 are very similar (Fig. 2). GST activi-





Figs. 1 and 2. Time course of glutathione transferase and glutathione peroxidase activities in rat liver cytosol from 2 to 16 days after a single i.p. treatment with Aroclor 1254 (500 mg/kg body wt) (Fig. 1) or fireMaster BP-6 (500 mg/kg body wt) (Fig. 2). On each day, the activities of two control and three pretreated rats from each group were determined. Enzyme activities for the seven control groups in total were 1402 ± 121 for CDNB (●), 45 ± 5.8 for EA (▲) and 33.6 ± 3.9 for AD (■) (nmoles/min/mg cytosolic protein) and 445 ± 65 for CHP (○), 305 ± 41 for *t*-BHP (△) and 269 ± 40 for H_2O_2 (□) (nmoles NADPH oxidized/min/mg cytosolic protein) ($\bar{X}$ ± S.D.). a or b denotes means which are significantly different from the control mean at $P \leq 0.01$ or $P \leq 0.05$, respectively.





Figs. 3 and 4. Effect of inducers on glutathione transferase (Fig. 3) and glutathione peroxidase (Fig. 4) activities in rat liver cytosol after pretreatment of the animals as described. Enzyme activities for the control groups were 1094 ± 216 for CDNB, 26.9 ± 3 for EA, 26.5 ± 4.3 for AD (nmoles/min/mg cytosolic protein) and 470 ± 61 for CHP, 394 ± 45 for *t*-BHP And 390 ± 66 for H_2O_2 (nmoles NADPH oxidized/min/mg cytosolic protein). Bars represent $\bar{X}$ ± S.D. for 3–4 rats. a or b denotes means which are significantly different from the control mean at $P \leq 0.01$ or $P \leq 0.05$, respectively.

ties were induced significantly with maxima at day 13 for CDNB (242% of control), at day 8 for both EA (253% of control) and AD (359% of control). Also the total glutathione peroxidase activity measured with CHP and *t*-BHP did not change significantly during the time course while the hydrogen peroxide-GSH-Px activity diminished significantly from day 6 to 16.

In a separate experiment the GST and GSH-Px activities were determined after treatment of the animals with various inducers as described in detail in Material and Methods. With all inducers the activity of GST towards CDNB and AD was significantly increased. The GST activity towards EA was significantly increased only in four cases: BHT, TSO, PB and (3,4)^{2}Cl (141, 193, 149 or 202% of control, respectively) (Fig. 3). In contrast to the highly inducible GST activities, GSH-Px activities were only slightly increased in three cases: activity towards CHP after treatment with ISO (128% of control) and PB (141% of control) and activity towards *t*-BHP after PB treatment (132% of control) (Fig. 4). GSH-Px activity with hydrogen peroxide was not increased by any treatment; there was even a significant decrease after treatment with TSO (69% of control).

DISCUSSION

The purpose of these studies was to investigate the regulation of GST and GSH-Px activities in rat liver cytosol. The administration of nine inducers clearly enhanced the activities of GST toward the broad-spectrum substrate CDNB. The two substrates, AD and EA, chosen because of their selectivity for GST isozymes which also possess peroxidase activity, however, showed differing effects. AD-GST activity was enhanced significantly by each of the GST inducers, while EA-activity was only slightly increased or remained unchanged. Expression of AD- and EA-GST activities may parallel the differential induction of mRNAs which code for the respective GST subunits. Pickett and co-workers [31] found that following PB administration the translatable mRNA for subunit 1 (Ya) was markedly increased while that for subunit 2 (Yc) was only slightly elevated. Likewise under conditions of selenium deficiency [20] or selenium excess [32] EA-GST activity varied independently. It is apparent that these activities, AD-GST and total GST on the one hand and EA-GST on the other, are under separate control.

The unexpected result that the potent GST inducers did not also generally enhance peroxidase activities cannot be explained simply on the basis that the poorly induced EA-GST isozymes contribute most of the non-Se-GSH-Px activity. Comparison of the *in vitro* activities reported here and the specific activities of the purified GST isozymes reported by Mannervik and Jensson [5] reveals that the EA- and AD-specific GST isozymes account for at least a third of the cytosolic GSH-Px activity (toward CHP). Therefore induction of these forms should have resulted in significant increases in GSH-Px activities, assuming that the induced isozymes in fact possess GSH-Px activity. An important unanswered question therefore is whether "B-type" GST isozymes isolated from rats which have been treated with potent GST inducers possess peroxidase activity.

The time course studies with the commercial products, Aroclor 1254 and fireMaster BP-6, demonstrate that, in contrast to the short-term induction studies mentioned above, all three GST activities were significantly increased with maxima from day 8 to day 13. Also here an increase in GSH-Px activities was not seen. In a preliminary report [33] we injected rats at various time intervals and killed them on a single day. In that experiment the observed increase in GST and the lack of increase in peroxidase activities may have been influenced by the fact that the rats were slightly different ages at the times of injection. In the experiment reported in this paper therefore we injected all the rats at a single time point, so that they would first be exposed to the xenobiotics at the same age, and killed them at various time points thereafter. Both experiments demonstrated qualitatively the same results, namely the potent induction of GST activities without a comcomitant increase in GSH-Px activities. Indeed a depression of Se-Px-activity (as measured by hydrogen peroxide), particularly evident during the second week, opens the possibility that animals exposed to polyhalogenated biphenyls may be impaired in their ability to withstand lipid peroxidative processes or other oxidative stresses. The recent observation that Se-GSH-Px activity was severely depressed following a lethal dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [34] suggests that this biochemical response may be generally involved in the toxicity of the polyhalogenated aromatic hydrocarbons. Future studies should be directed at uncovering the mechanism of Se-GSH-Px depression following exposure to these and related compounds and delineating the involvement of this biochemical response in their toxicity.

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REFERENCES

1. R. A. Lawrence and R. F. Burk, *J. Nutr.* **108**, 211 (1978).
2. *Functions of Glutathione in Liver and Kidney* (Eds. H. Sies and A. Wendel). Springer, Berlin (1978).
3. R. A. Lawrence, L. K. Parkhill and R. F. Burk, *J. Nutr.* **108**, 981 (1978).
4. M. E. Tappel, F. Chaudiere and A. L. Tappel, *Comp. Biochem. Physiol.* **73B**, 945 (1982).
5. B. Mannervik and H. Jensson, *J. biol. Chem.* **257**, 9909 (1982).
6. C.-P. D. Tu, H.-C. J. Lai, N. Li, M. J. Weiss and C. C. Reddy, *J. biol. Chem.* **259**, 9434 (1984).
7. W. B. Jakoby, B. Ketterer and B. Mannervik, *Biochem. Pharmac.* **33**, 2539 (1984).
8. D. J. Meyer, L. G. Christodoulides, H. Tan and B. Ketterer, *FEBS Lett.* **173**, 327 (1984).
9. G. Clifton and N. Kaplowitz, *Biochem. Pharmac.* **27**, 1284 (1978).
10. T. Friedberg, P. Bentley, P. Stasiecki, H. R. Glatt, D. Raphael and F. Oesch, *J. biol. Chem.* **254**, 12028 (1979).
11. J. S. Felton, J. W. Ketley, N. B. Jakoby, A. Aitio, J. R. Bend and D. W. Nebert, *Molec. Pharmac.* **18**, 559 (1980).
12. J. W. DePierre, J. Seidegard, R. Morgenstern, L. Balk, J. Meijer, A. Aström, J. Norelius and L. Ernster. *Xenobiotica* **14**, 295 (1984).
13. C. Guthenberg, R. Morgenstern, J. W. DePierre and B. Mannervik, *Biochem. biophys. Acta* **631**, 1 (1980).
14. P. DiSimplicio, H. Jensson and B. Mannervik, *Acta Chem. Scand.* **B37**, 255 (1983).
15. T. N. Thompson, J. B. Watkins, Z. Gregus and C. D. Klaasen, *Toxicol. appl. Pharmac.* **66**, 400 (1982).

16. J. Marniemi, M. Nokkala, H. Vainio and K. J. W. Hartiala, *Chem.-Biol. Interact.* **18**, 247 (1977).
17. A. J. Baars, M. Jansen and D. D. Breimer, *Biochem. Pharmac.* **27**, 2487 (1978).
18. L. Dock, M. Martinez and B. Jernström, *Carcinogenesis* **5**, 841 (1984).
19. T. Masukawa, T. Nishimura and H. Iwata, *Biochem. Pharmac.* **33**, 2635 (1984).
20. R. Reiter and A. Wendel, *Biochem. Pharmac.* **33**, 1923 (1984).
21. Y. C. Awasthi, C. A. Partridge and D. D. Dao, *Biochem. Pharmac.* **32**, 1197 (1983).
22. F. S. Kawahara, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 5, p. 527. Academic Press, New York (1962).
23. F. L. W. van Roosmalen, *Rec. Trav. Chim.* **53**, 359 (1934).
24. S. Safe and O. Hutzinger, *J. Chem. Soc. Perkin* **I**, 686 (1972).
25. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
26. D. E. Paglia and W. N. Valentine, *J. Lab. & Clin. Med.* **70**, 158 (1967).
27. A. Wendel, in *Methods in Enzymology* (Ed. W. B. Jacoby), Vol. 77, p. 325. Academic Press, New York (1981).
28. W. H. Habig, M. J. Pabst and W. B. Jacoby, *J. biol. Chem.* **249**, 7130 (1974).
29. A. M. Benson, P. Talalay, J. H. Keen and W. B. Jacoby, *Proc. natn. Acad. Sci.* **74**, 158 (1977).
30. C. W. Dunnett, *Biometrics* **6**, 482 (1964).
31. C. B. Pickett, A. M. Donohue, A. Y. H. Lu and B. F. Hales, *Archs Biochem. Biophys.* **215**, 539 (1982).
32. R. Reiter and A. Wendel, *Biochem. Pharmac.* **34**, 2287 (1985).
33. L. W. Robertson, J. Merrill, I. Lambert and S. Safe, *The Toxicologist* **3**, 100 (1983).
34. S. J. Stohs, M. Q. Hassan and W. J. Murray, *Xenobiotica* **14**, 533 (1984).