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SPECIES AND ORGAN SPECIFICITY OF THE TRANS-STILBENE OXIDE INDUCED EFFECTS ON EPOXIDE HYDRATASE AND BENZO(A)PYRENE MONOOXYGENASE ACTIVITY IN RODENTS

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Abstract—It was investigated, whether the selective induction of epoxide hydratase by *trans*-stilbene oxide (TSO) represents a general phenomenon or is confined to the liver of male rats where it was discovered. Therefore the effect of treatment with TSO on epoxide hydratase and benzo(a)pyrene monooxygenase activities were investigated in other organs (kidney, lung, skin, testis), other species (mice, hamsters) and also in female rats. In female rat livers the effect of TSO on the measured enzyme activities was very similar to that found in the male rat liver, i.e. a large induction of epoxide hydratase activity to 300–400 per cent of controls without affecting the benzo(a)pyrene monooxygenase activity. The potency of TSO to induce liver epoxide hydratase activity expressed as per cent of controls was 350:180:140 in rat, mouse and hamster, respectively. Selective induction of epoxide hydratase was found in rat and hamster liver, but not in the mouse liver, where benzo(a)pyrene monooxygenase activity was induced to about the same extent as the epoxide hydratase activity. The only extrahepatic organ in which an increased epoxide hydratase activity was found after TSO treatment was the rat kidney. Subcutaneous and topical treatment with TSO for 12 and 10 days respectively did not induce rat skin epoxide hydratase activity, instead a decrease of the enzyme activity to about 70 per cent of that found in control animals was found. Thus, TSO which was demonstrated to be a selective inducer of epoxide hydratase in rat liver can be utilized so far only in a limited number of carcinogenicity test systems, since it failed to induce the skin epoxide hydratase activity, which would have been an excellent tool to study directly the role of epoxide hydratase in the mechanism of skin tumor formation caused by polycyclic hydrocarbons. Interestingly, the epoxide hydratase of the hamster, investigated for the first time in this study, proved quite different from that of rat and mouse in that it hydrated styrene oxide remarkably faster than benzo(a)pyrene 4,5-oxide. This was true for all organs investigated. Also, the organ distribution of epoxide hydratase proved to be very different from that in rat and mouse. In the mouse the activity (with benzo(a)pyrene 4,5-oxide as substrate) was amongst all organs investigated highest in the testis (2.5 fold as compared to liver) but in the hamster the activity was more than 100 fold lower in testis as compared to liver. On the other hand, the activity in kidney was about 50 fold higher in hamster as compared to mouse.

The microsomal monooxygenase system and epoxide hydratase (epoxide hydrase), both widely distributed in mammalian organs [1, 2], are important enzymes in the metabolism of carcinogenic polycyclic hydrocarbons [3–9]. The monooxygenase system catalyses the oxidative biotransformation of a great number of endogenous and exogenous compounds [10, 11] and is also responsible for the formation of electrophilically reactive epoxides from compounds bearing an olefinic or aromatic moiety [3–9]. Epoxides may covalently bind to cellular macromolecules such as DNA, RNA and protein and may thereby exert a cytotoxic, mutagenic or carcinogenic effect [3–9]. The inactivation of such reactive epoxide intermediates can be catalysed by the cytoplasmic glutathione-*S*-transferases [12–15] and by the microsomal epoxide hydratase [4–6]. The latter enzyme may be quite critical in this respect, since it is localised in the same subcellular fraction as the epoxide-producing monooxygenase system. However, this inactivating function of epoxide hydratase became questionable, when it was discovered [16–21] that the same enzyme may also in certain cases provide the precursor dihydrodiols for highly mutagenic dihydrodiol epoxides such as 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene 9,10-oxide which cannot be inactivated by epoxide hydratase. To investigate the *in vivo* role of epoxide hydratase in the metabolism of polycyclic hydrocarbons to mutagenic and carcinogenic species, we have developed a selective epoxide hydratase inducer [22, 23]. *trans*-Stilbene oxide (TSO) was found to be a potent and selective inducer of epoxide hydratase activity in rat liver with no effect on 5 investigated monooxygenase parameters [23]. In fact 12 hr after a single dose of TSO the monooxygenase activities were inhibited, but they were neither increased nor inhibited at any other time point up to 12 days including the time points of maximal epoxide hydratase induction at 2 and 3 days after treatment, nor at any of the investigated doses of TSO. Therefore it was concluded that the epoxide hydratase induction is selective indeed and not only apparently so, resulting

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from a compensation of a possible induction of monooxygenase activity by an inhibition by TSO or its metabolites which would have to be of the same extent as the induction at all doses and time points investigated [23].

In this study the effect of TSO treatment on epoxide hydratase activity in liver, kidney and lung as well as on liver benzo(a)pyrene monooxygenase activity of female rats, male NMRI mice and male Syrian Golden hamsters was compared to the effects observed in male rats, in order to see whether there are any sex or species differences and thereby possibly detect an induction of epoxide hydratase in the lung of one of these species. Moreover, results on the effect of subcutaneous and topical treatment with TSO on rat skin epoxide hydratase and benzo(a)pyrene monooxygenase activity are presented.

MATERIALS AND METHODS

Chemicals. trans-Stilbene oxide was purchased from EGA- Chemie, Steinheim, West Germany and was purified by treatment with charcoal in ether and recrystallisation from benzene/methanol. The purity was established by thin-layer chromatography in ether and chloroform/petroleum ether (1:1). Also, no depression of the melting point was noted. [^{3}H]benzo(a)pyrene 4,5-oxide was prepared according to the method of Dansette and Jerina [24] under conditions as described in [25]. [G-^{3}H]benzo(a)pyrene (5 Ci/mmole) was obtained from the Radiochemical Centre, Amersham, England. [7-^{3}H]Styrene oxide was prepared as described [27]. NADPH, NADH and glucose-6-phosphate were purchased from Serva, Heidelberg, West Germany; glucose-6-phosphate dehydrogenase from Boehringer Mannheim, West Germany; and 2-(4-biphenyl)-5-(*p*-t-butyl-phenyl-1,3,4-oxa-diazol (butyl PBD) from Zinsser, Frankfurt a.M., West Germany. Other chemicals were of the purest grade commerically available.

Animal treatment. Adult male and female Sprague–Dawley rats (180–280 g) were obtained from Versuchstierzuchtanstalt WIGA, Sulzfeld, West Germany and male Syrian Golden hamsters (80–100 g) from Tierhandlung Walter, Worms, West Germany. Male NMRI mice (25–35 g) were a gift from Professor E. Pfeiffer from the Institute of Hygiene, University Mainz. Animals were kept under standardized conditions as described [22] and were always treated between 8 and 10 a.m. using dosage schedules as indicated in legends to Tables.

Preparation of microsomes. The animals were killed between 8 and 10 a.m. by a blow on the head and cervical dislocation. Microsomes from liver, kidney, lung and testis were prepared as described [22]. Skin microsomes were prepared using an Ultraturrax homogenizer as described [25]. Protein concentrations were determined by the method of Lowry *et al.* [26] with bovine serum albumin as standard.

Enzyme assays

General. Determination of epoxide hydratase and benzo(a)pyrene monooxygenase activity was performed with freshly prepared microsomes, which were kept in an ice bath and were used within 36 hr after killing the animals. No measurable losses of these enzyme activities were found, when microsomes were kept on ice for 48 hr.

The enzyme assays proceeded linearly with respect to the protein concentrations and incubation times used.

Epoxide hydratase. Epoxide hydratase activity was determined using the radiometric extraction assays with [^{3}H]benzo(a)pyrene 4,5-oxide [25] and [7-^{3}H]styrene oxide [27] as substrates. The latter assay was performed under conditions described in [28] (in the absence of Tween 80). Enzyme activities are corrected for 80 per cent recovery of benzo(a)-pyrene-4,5-dihydrodiol and 86 per cent recovery of styrene glycol.

Benzo(a)pyrene monooxygenase. The benzo(a)pyrene monooxygenase activity (also called "aryl hydrocarbon hydroxylase") was measured by fluorimetric determination of the phenolic benzo(a)pyrene products using 3-hydroxybenzo(a)pyrene and quinine sulfate as standards, essentially as described by Nebert and Gelboin [29] under conditions as described [22].

RESULTS AND DISCUSSION

Effect of trans-*stilbene oxide (TSO) treatment on epoxide hydratase and benzo(a)pyrene monooxygenase activity in the liver of male and female rats, male NMRI mice and male Syrian Golden hamsters*

TSO was demonstrated to selectively induce epoxide hydratase activity in rat liver without affecting the cytochrome P450 content, λ_{max} of the reduced CO-complex and the activities of aminopyrine *N*-demethylase and benzo(a)pyrene monooxygenase when determined fluorimetrically and radiometrically [23]. It was now checked, whether epoxide hydratase can also be selectively induced by TSO in the liver of female rats as well as in the mouse and hamster liver. As parameter for the monooxygenase system the benzo(a)pyrene monooxygenase activity was chosen, since it represents the most relevant enzyme activity with respect to this project. As can be seen from Table 1 the effect of TSO treatment on the enzyme activities in female rat liver was of the same order of magnitude as observed in male rat liver. The epoxide hydratase activity was induced to about 150 per cent and 300–400 per cent of controls after treatment with the low and the high dose of TSO respectively. In the liver of NMRI mice the epoxide hydratase and the benzo(a)pyrene monooxygenase activities behave quite differently to those in the rats upon treatment with TSO. The epoxide hydratase activity was induced to only 182 per cent of the control activity after treatment with daily doses of 2.0 mmol TSO per kg body wt for 3 days and the benzo(a)-pyrene monooxygenase activity was also induced to about the same extent (155 per cent of controls). It is noteworthy that in the hamster liver, in contrast to the rat and various mouse strains [30] the specific activities of epoxide hydratase were very different when determined with benzo(a)pyrene 4,5-oxide or styrene oxide as substrates and control activities were about 2 and 5 fold higher than those found in male rat liver microsomes. TSO had only a small inducing effect on hamster liver epoxide hydratase

Table 1. Effect of *trans*-stilbene oxide (TSO) on epoxide hydratase and benzo(a)pyrene monooxygenase activity in the liver of rats, mice and hamsters

	mmol TSO/kg*	Epoxide hydratase†		Benzo(a)pyrene monooxygenase†
		Benzo(a)pyrene 4,5-oxide	Styrene oxide	
Female rats	0	3.67 ± 0.37	4.40 ± 0.56	48 ± 8
	0.2	6.23 ± 0.84 (170)§	6.90 ± 1.12 (157)‡	52 ± 5 (108)
	2.0	14.79 ± 2.43 (403)§	14.77 ± 2.63 (336)§	59 ± 7 (123)
Male rats	0	5.71 ± 0.81	7.32 ± 0.54	310 ± 35
	0.2	8.39 ± 1.33 (147)‡	11.20 ± 1.50 (153)‡	344 ± 22 (111)
	2.0	16.86 ± 1.12 (295)§	23.12 ± 2.01 (316)§	332 ± 32 (108)
Male NMRI mice	0	1.02 ± 0.12	n.d.	296 ± 40
	0.2	1.48 ± 0.16 (145)‡	n.d.	356 ± 26 (121)‡
	2.0	1.85 ± 0.26 (182)§	n.d.	458 ± 39 (155)§
Male hamsters	0	11.73 ± 1.27	35.9 ± 2.11	1351 ± 185
	0.2	11.76 ± 1.86 (100)	36.6 ± 3.20 (102)	1457 ± 91 (108)
	2.0	16.26 ± 2.29 (139)‡	52.6 ± 5.20 (146)§	1437 ± 172 (106)

* Male and female Sprague–Dawley rats (180–280 g), male NMRI mice (25–35 g) and male Syrian Golden hamsters (80–100 g) were treated by a daily intraperitoneal injection of the indicated dose of TSO per kg body wt for 3 days and were killed 24 hr after the last injection. TSO was dissolved in sunflower oil. Control animals received the appropriate volume of vehicle alone (0.5 ml for the rats and 0.3 ml for the mice and hamsters).

† Epoxide hydratase activities are expressed as nmol benzo(a)pyrene-4,5-dihydrodiol or styrene glycol, respectively, formed per mg microsomal protein per min. Benzo(a)pyrene monooxygenase activities are expressed as pmol 3-hydroxybenzo(a)pyrene per mg microsomal protein per min. The results represent the means ± S.D. from 3 individual experiments which were performed on microsomes from pooled organs of 3–6 animals per group. The assays were carried out in duplicates for each of 2 protein concentrations. Standard deviations of the means of single determinations were usually less than 7 per cent for the epoxide hydratase assays and less than 10 per cent for the benzo(a)pyrene monooxygenase assay. Numbers in parenthesis give the enzyme activities as percent of control activities.

Students '*t*' test was used to establish the significance of differences between the means. $P < 0.05$ (‡), $P < 0.005$ (§).

activity (about 140 per cent of controls) after daily treatment with 2.0 mmol TSO per kg body wt for 3 days. No effect on the liver benzo(a)pyrene monooxygenase activity was observed in this species after TSO treatment.

Since nothing is known about the mechanism of epoxide hydratase induction caused by TSO, apart from its dependency on RNA and protein synthesis in rat liver [23], we cannot explain these species differences. Assuming that TSO is metabolised by the monooxygenase system, which is certainly the case, one might try to explain these species differences by a different ability of the liver monooxygenase system to activate TSO to a hypothetic ultimate inducer or else to inactivate TSO as inducer and accelerate its excretion. However, this possibility appears rather unlikely, since the known sex difference in the rat with respect to the monooxygenase system (e.g. a six-fold higher benzo(a)pyrene monooxygenase activity in male than in female rats) had apparently no effect on the extent of the epoxide hydratase induction. Moreover, a combined treatment of male rats with TSO together with two different types of monooxygenase inducers (phenobarbital and β-naphthoflavone) did not result in a significantly increased epoxide hydratase activity as compared to that observed after treatment with TSO alone (unpublished results). One reasonable explanation for these species differences, amongst many others, would be a different affinity of TSO to the receptor(s) responsible for epoxide hydratase induction. The receptor(s) in male and female Sprague–Dawley rats would then be very similar, but different from that (those) in NMRI-mice and Syrian Golden hamsters.

Effect of trans*-stilbene oxide (TSO) treatment on epoxide hydratase activity in kidney and lung of male and female rats, male mice and male hamsters*

Firstly one should take notice of the extreme differences of the control activities in these organs. Whereas male and female rats showed about the same specific epoxide hydratase activity in kidney or in lung, when determined with both substrates, there were considerable variations between the species. The hamster possesses a 50-fold higher kidney epoxide hydratase activity than the NMRI mouse when determined with benzo(a)pyrene 4,5-oxide as substrate. Using styrene oxide as substrate the epoxide hydratase activity in kidney of NMRI mice was too low to be accurately determined and must therefore have been lower than 0.1 nmol per mg protein per min, which results in at least a 100-fold difference between hamster and mouse kidney epoxide hydratase activity towards this substrate (Table 2).

With respect to epoxide hydratase induction caused by TSO, rat kidney was the only responsive extrahepatic organ detected so far amongst the species investigated. However, the extent of induction was lower than in the liver. After treatment with the low dose of TSO there was no increase in rat kidney epoxide hydratase activity, whereas in the liver the same dose caused a 50–70 per cent increase of the enzyme activity. After treatment with daily doses of

Table 2. Effect of *trans*-stilbene oxide (TSO) treatment on epoxide hydratase activity in the kidney of rats, mice and hamsters

	mmoles TSO/kg*	Epxoide hydratase†	
		BP 4,5-oxide	Styrene oxide
Female rats	0	0.51 ± 0.06	0.65 ± 0.06
	0.2	0.50 ± 0.04 (98)	0.57 ± 0.03 (88)
	2.0	1.21 ± 0.17 (242)§	1.80 ± 0.22 (277)§
Male rats	0	0.59 ± 0.11	0.73 ± 0.08
	0.2	0.64 ± 0.08 (108)	0.74 ± 0.06 (101)
	2.0	0.95 ± 0.07 (161)§	1.10 ± 0.11 (151)§
Male NMRI mice	0	0.071 ± 0.011	< 0.1
	0.5	0.073 ± 0.008 (103)	< 0.1
	2.0	0.079 ± 0.013 (113)	< 0.1
Male hamsters	0	3.52 ± 0.41	8.71 ± 0.95
	0.5	3.45 ± 0.10 (98)	9.36 ± 1.01 (107)
	2.0	3.88 ± 0.36 (110)	9.90 ± 0.99 (114)

*, †, § see Table 1.

2.0 mmol TSO per kg body wt for 3 days, epoxide hydratase activity was induced in the kidney of male and female rats. A marked sex difference was observed in that the induction in the kidney was considerably higher in female rats (about 260 per cent of controls) than in male rats (about 155 per cent of controls). In the kidney of NMRI mice and hamsters TSO caused no significant changes in epoxide hydratase activity.

The hope of finding an induction of epoxide hydratase activity in the lung of one of these species was not fulfilled. Instead, if there was any effect of TSO, the enzyme activity was slightly decreased (Table 3).

Effect of trans*-stilbene oxide (TSO) treatment on epoxide hydratase activity in the testis of mice and hamsters*

In a recent study on the distribution of epoxide hydratase in organs of the rat and mouse, epoxide hydratase activity was found to be surprisingly high in the testis [2]. Therefore it was suggested that epoxide hydratase may be involved in steroid metabolism. Since TSO has a similar shape to the synthetic oestrogen diethylstilbestrol it was interesting to see whether it affects the epoxide hydratase activity in the testis. However, no effect of TSO on testis epoxide hydratase activity was observed apart from a slight reduction in hamster testis. Interestingly the epoxide hydratase activity in the hamster testis was about 25-fold lower than that in mouse testis. This contrasts with the situation in the kidney and lung, where epoxide hydratase activity was 50 to 10 fold higher in the hamster than in the mouse. Whilst in the rat epoxide hydratase specific activity (determined with benzo(a)pyrene 4,5-oxide as substrate) was, immediately after the liver, second highest in testis amongst all 26 organs and tissues investigated [2] and in the mouse even higher (2.5-fold) than in the liver [2], the present study shows that in hamster it is more than 100-fold lower in testis than in liver and more than 30-fold lower than in kidney and lung. The benzo(a)pyrene monooxygenase in testis showed about the same activity (3–4 pmol 3-hydroxybenzo(a)pyrene per mg microsomal protein per min) in both species.

These extreme differences in the distribution pattern of epoxide hydratase activity in rat, mouse and hamster may possibly correlate with the endogenous function of epoxide hydratase. However, since such an endogenous function of this enzyme is still unknown, we cannot explain this phenomenon.

Table 3. Effect of *trans*-stilbene oxide (TSO) treatment on epoxide hydratase activity in the lung of rats, mice and hamsters

	mmol TSO/kg*	Epoxide hydratase†	
		BP 4,5-oxide	Styrene oxide
Female rats	0	0.28 ± 0.04	0.47 ± 0.06
	2.0	0.27 ± 0.03 (96)	0.36 ± 0.05 (77)‡
Male rats	0	0.36 ± 0.03	0.42 ± 0.03
	2.0	0.30 ± 0.04 (83)	0.35 ± 0.05 (83)
Male NMRI mice	0	0.37 ± 0.04	n.d.
	0.5	0.36 ± 0.04 (97)	n.d.
	2.0	0.37 ± 0.02 (100)	n.d.
Male hamsters	0	3.67 ± 0.22	10.36 ± 0.81
	0.5	3.88 ± 0.41 (106)	8.99 ± 1.11 (87)
	2.0	3.47 ± 0.45 (95)	8.15 ± 0.87 (79)‡

*, †, ‡ see Table 1.

Table 4. Effect of *trans*-stilbene oxide (TSO) treatment on epoxide hydratase activity in the testis of mice and hamsters

	mmol TSO/kg*	Epoxide hydratase† BP 4,5-oxide	Styrene oxide
NMRI mice	0	2.54 ± 0.31	n.d.
	0.5	2.85 ± 0.18 (112)	n.d.
	2.0	2.56 ± 0.22 (101)	n.d.
Hamsters	0	0.107 ± 0.008	0.279 ± 0.031
	0.5	0.096 ± 0.011 (90)	0.240 ± 0.025 (86)
	2.0	0.094 ± 0.006 (88)‡	0.205 ± 0.020 (73)‡

*, †, ‡ see Table 1.

Effect of subcutaneous and topical treatment with trans-*stilbene oxide (TSO) on rat skin and liver epoxide hydratase and benzo(a)pyrene monooxygenase activity*

Rat skin is susceptible to benzo(a)pyrene induced tumors [31, 32]. Therefore, selective induction of epoxide hydratase activity in skin would provide a useful tool to clarify whether epoxide hydratase has a predominantly activating or inactivating function in the tumor formation caused by polycyclic hydrocarbons. However, whereas rat liver epoxide hydratase activity was increased to 182 and 212 per cent of controls after subcutaneous treatment with TSO, skin epoxide hydratase activity in the same rats was decreased to 66 and 79 per cent of controls (Table 5). As expected the benzo(a)pyrene monooxygenase activity in the liver was unchanged after subcutaneous treatment during 12 days using dosage schedules as indicated in the legend to Table 5. Although the benzo(a)pyrene monooxygenase activity in the rat skin microsomes was at the limit of detectability, we can say that, if there was any effect, the benzo(a)-pyrene monooxygenase activity was slightly decreased. Since the lack of an induction of epoxide hydratase activity in the skin after subcutaneous treatment might have been the result of a rapid absorption of TSO, attempts were also made to induce the enzyme by topical application of TSO for 10 days. However, the results were very similar to those after subcutaneous treatment. One might think of an inhibition of epoxide hydratase by the presence of TSO in the skin microsomes. However, TSO is a very weak inhibitor of epoxide hydratase in skin [33]. Moreover, incubation of mixtures of control skin microsomes together with various amounts of skin microsomes from TSO treated rats always resulted in enzyme activities corresponding to the sum of the two components. Thus, these results suggest that the reduced epoxide hydratase activity in rat skin is due to a repression rather than to an inhibition of the enzyme by TSO.

In summary, when different species were treated with TSO and the effect on epoxide hydratase and

Table 5. Effect of subcutaneous and topical treatment with *trans*-stilbene oxide (TSO) on rat skin and liver epoxide hydratase and benzo(a)pyrene monooxygenase activity

	Dosage schedule*	Mode of application	Epoxide† hydratase	Benzo(a)pyrene† monooxygenase
Skin	oil	s.c.	134 ± 35	< 1
	A	s.c.	89 ± 1 (66)	< 1
	B	s.c.	106 ± 4 (79)	< 1
	oil	topical	151 ± 6	< 1
	C	topical	98 ± 8 (65)‡	< 1
Liver	oil	s.c.	6860 ± 480	283 ± 44
	A	s.c.	12470 ± 2340 (182)§	267 ± 29 (94)
	B	s.c.	14530 ± 1270 (212)§	280 ± 11 (99)

* Male Sprague–Dawley rats (200–280 g) were treated using the following dosage schedules: A. Animals received subcutaneous injections of 2 mmol TSO per kg body wt in 0.5 ml sunflower oil on day 1, 2, 3, 7 and 11. B. The same dose was used, but was injected on day 1, 2, 3, 5, 7, 9 and 11. The animals of group A and B were killed 48 hr after the last injection. C. Rats were shaved on the neck (area about 4 × 6 cm). Animals were kept individually and received a daily topical application of 2 mmol TSO per kg body wt dissolved in 0.25 ml of dimethylsulfoxide for 10 days. The animals were killed 24 hr after the last application.

† Epoxide hydratase activities are expressed as pmol benzo(a)pyrene 4,5-dihydrodiol per mg microsomal protein per min. Benzo(a)pyrene monoxygenase activities are expressed as pmol 3-hydroxybenzo(a)pyrene per mg microsomal protein per min. Assays were performed using individual microsomal preparations of skin from 2–3 animals and of the liver from 4 animals. Duplicate determinations were done for each of 2 protein concentrations of liver and of 3 protein concentrations of skin microsomes.

Numbers in parenthesis give the enzyme activities as per cent of control activities. Using the student's '*t*' test, the significance of differences between the means was: $P < 0.05$ (‡), $P < 0.005$ (§).

benzo(a)pyrene monooxygenase activity in various organs of these species was investigated, it became apparent that the effect of TSO on these enzyme activities may vary considerably between species and organs. It might be argued that the kinetics of induction could be different from species to species and from organ to organ. However, since we treated the animals daily for 3 days with TSO and measured the enzyme activities 24 hr after the last treatment, an epoxide hydratase induction not detected in this system would have to be either extremely short-lived (much shorter than 24 hr as opposed to much longer than 6 days in the male rat liver) or must have a very unusually long-lasting lag period before onset of induction (much longer than 4 days as opposed to less than 12 hr in the male rat liver).

Marked differences between the species were also observed in the basal epoxide hydratase activities in the various organs investigated, but no correlation between the basal activity and inducibility of epoxide hydratase by TSO was found. The potency of TSO to induce liver epoxide hydratase in the various species declines in the order Sprague–Dawley rat > NMRI mouse > Syrian Golden hamster. Benzo(a)-pyrene monooxygenase activity in male and female rat liver as well as in hamster liver was not affected by TSO treatment, but in the liver of NMRI mice, it was induced to about the same extent as the epoxide hydratase activity. The only extrahepatic organ in which the epoxide hydratase activity was increased after TSO treatment was the kidney, but only in the rats and not in mice and hamsters. In rat skin the epoxide hydratase activity was even decreased to about 70 per cent of controls after subcutaneous or topical treatment with TSO. Thus the range of application of TSO as a diagnostic tool for investigations on the role of epoxide hydratase in the overall metabolism of polycyclic hydrocarbons to mutagenic and carcinogenic species is limited to systems where rat liver can be used. Based on these results, further investigations are in progress to find a selective skin epoxide hydratase inducer.

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