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# Regulation of 3,3′,4,4′-Tetrachlorobiphenyl Induced Cytochrome P450 Metabolism by Thiols in Tissues of Rainbow Trout*

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**ABSTRACT.** We observed that glutathione (GSH) status regulates the Ah receptor inducible cytochrome P4501A (CYP1A) gene expression and catalytic activity in 3,3′,4,4′-tetrachlorobiphenyl (TCB) exposed rainbow trout. Tissue GSH status of TCB (1 mg/kg body weight, in corn oil) injected fish was manipulated by a) injecting (i.p.) GSH (0.25 g/kg), b) arresting GSH synthesis by L-buthionine-[S,R]-sulfoximine (BSO; 6 mmol/kg) injection for 3 and 6 days. Our attempt to manipulate GSH levels by lipoate supplementation (16 mg/kg) was not productive. Both BSO- and lipoate-supplemented fish maintained a low tissue redox (GSSG/GSH) ratio. Activities of glutathione peroxidase and glutathione reductase were elevated following 3 days of GSH supplementation in GSH rich tissues. Low activities of these enzymes were observed in BSO treated GSH deficient tissues. TCB injection markedly induced hepatic and renal CYP1A catalytic (ethoxyresorufin O-deethylase [EROD]) activities. This effect was further potentiated (3-fold) in GSH-supplemented fish tissues. In contrast, EROD induction by TCB was markedly suppressed in GSH deficient (BSO-treated) and lipoate-supplemented fish. The suppression of CYP1A catalytic activities in GSH deficient and lipoate-supplemented fish was consistently associated with a suppression of TCB induced CYP1A mRNA and protein expressions in these groups. In glutathione-supplemented fish, TCB induced CYP1A protein expression was markedly higher following 3 days of GSH supplementation. Results of our study suggest that tissue thiol status modulates cytochrome P450 CYP1A gene expression and catalytic activity. COMP BIOCHEM PHYSIOL 117C;3:299–309, 1997. © 1997 Elsevier Science Inc.

**KEY WORDS.** Glutathione, *Ah* gene battery, cytochrome P4501A, α-lipoic acid, redox, polychlorinated biphenyls, pollution, *Oncorhynchus mykiss*

## INTRODUCTION

Polychlorinated biphenyls (PCBs) are major industrial pollutants which have been heavily produced in several industrial settings. Their production has now discontinued, but PCBs remain a worldwide pollution problem (1). The effects of toxicities of various PCB congeners to terrestrial and aquatic organisms are wide ranging causing physical and biochemical changes including activation of xenobiotic metabolism in mammals and fish (2–4). PCBs share many properties with polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, including some of the most toxic chemicals known such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (1,5).

The xenobiotic responses predominantly induced by PCB congeners in mammals and fish are phase I and II enzyme activities (3,4,6). Phase I enzymes are almost exclusively cytochromes P450, while phase II enzymes usually function by conjugating phase I metabolites to, e.g., glutathione or glucuronide to facilitate the excretion of a xenobiotic (7). Pollutant induced cytochrome P450 enzymes have been characterized in various fish species (8–11). Induction of these enzymes in fish as in mammals is thought to occur through ligand (e.g., TCDD) binding to the Ah receptor (AhR), translocation, and transformation of this complex in the nucleus involving ARNT (Ah receptor nuclear trans-

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*Abbreviations*–AhR, aromatic hydrocarbon receptor; AhRE, aromatic hydrocarbon responsive element; ARNT, aromatic receptor nuclear translocator; ATCC, America Type Culture Collection; BSO, L-buthionine-[S,R]-sulfoximine; CYP1A, cytochrome P4501A; cDNA, complimentary deoxyribonucleic acid; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; EDTA, ethylenediaminetetraacetic acid; EROD, ethoxyresorufin O-deethylase; EPRE, electrophilic responsive element; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; mRNA, messenger ribonucleic acid; MS 222, 3-aminobenzoic acid ethyl ester; PAGE, polyacrylamide gel electrophoresis; PCBs, polychlorinated biphenyls; RNA, ribonucleic acid; SSC, saline sodium citrate; SDS, sodium dodecylsulfate; TCB, 3,3′,4,4′-tetrachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TGSH, total glutathione; UDPGT, uridine diphosphate glucuronosyltransferase.

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locator) protein, and ultimately DNA binding of the complex to the aromatic hydrocarbon responsive element (AhRE) (12,13). Such DNA binding induces the *Ah* gene battery, which consists in mammals of at least six genes, two phase I (CYP1A1 and CYP1A2) and four phase II genes, which include uridine diphosphate-(UDP) glucuronosyltransferase (UDPGT) and one glutathione S-transferase form (7). In fish, the main focus has been on phase I induction mechanisms, and two distinct CYP1A genes have been described in rainbow trout (11).

A phylogenetic analysis of CYP1A genes in vertebrates has been presented and suggests an early appearance in vertebrate evolution (10). In spite of a better knowledge of the function of the Ah receptor, little is known of the physiological conditions that regulate the *Ah* gene battery. Nebert *et al.* (7) proposed that the *Ah* gene battery responds to oxidative stress. It was recently shown that the *in vitro* DNA binding of the Ah receptor is redox regulated (14).

PCBs are known to perturb the balance between cellular pro-oxidant and antioxidant forces in vertebrates and thus induce oxidative stress (15,16). Reduced glutathione (L-$\gamma$-glytamyl-L-cysteinylglycine, GSH) is a major cellular antioxidant with numerous key functions. Glutathione S-transferase (GST) catalyzes the conjugation of the sulfhydryl group of GSH and an electrophilic atom of a xenobiotic compound. Glutathione-dependent antioxidant defenses are catalyzed by glutathione peroxidase (GPX), which decomposes hydrogen peroxide and organic peroxides generating oxidized glutathione (GSSG) as endproduct. The tissue GSH pool is maintained in its reduced state by the action of glutathione reductase (GR) at the expense of NADPH. Other antioxidants including vitamins E and C and $\alpha$-lipoic acid, act synergistically with glutathione and have often been used in antioxidant supplementation studies (17,18). Although vitamins E and C are powerful antioxidants and their tissue availability can be enhanced by oral supplementation, their beneficial antioxidant actions involve radical byproducts which are reduced by GSH and lipoate (17,18). Increasing the cellular thiol pool (primarily established by -SH either as GSH or protein bound -SH) has been a challenging task in mammalian studies. Administration of GSH to rats was shown to increase GSH levels in the intestinal mucosa (19), but i.p. injections of GSH failed to increase tissue GSH concentrations (20). Supplementation of lipoate as an effective pro-GSH agent has become a more successful approach (18,21).

3,3′,4,4′-Tetrachlorobiphenyl (TCB) influences xenobiotic metabolism and GSH-dependent antioxidant defenses in rainbow trout (4). Yet there is no evidence that alteration of the cellular thiol pool concomitantly interferes with the expression and activity of phase I enzymes. The arrest of tissue glutathione synthesis by L-buthionine-[S,R]-sulfoximine (BSO) is well established to study the biochemical adaptation in mammals in a glutathione deficient and thus oxidative stress susceptible state (22). GSH deficiency induces the expression of phase II genes in a non-Ah receptor mediated fashion (23), and Ireland *et al.* (14) proposed that redox conditions regulate the DNA binding of the Ah receptor. These data suggest a possible influence of GSH on *Ah* battery genes, but no effect on CYP1A expression has yet been reported. The importance of CYP1A genes in xenobiotic metabolism warrants a better understanding of the physiological conditions that modulate the expression of these genes and their related enzymatic activities. It was our objective to examine the inducibility of Ah battery enzymes in TCB exposed trout, which were further subjected to manipulation of tissue glutathione status. We evaluated the role of two different pro-GSH agents (lipoate and GSH) versus GSH depletion in TCB induced P450 metabolism and present novel evidence that tissue thiol status regulates cytochrome P4501A expression.

## MATERIALS AND METHODS

### *Chemicals*

3,3′,4,4′-Tetrachlorobiphenyl was purchased from Ultra Scientific (Kingstown, RI). Ethoxyresorufin, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), bakers yeast GR, GSH, GSSG, BSO, $\alpha$-lipoic acid, 1 chloro-2,4-dinitrobenzene, NADPH, uridine 5′diphosphoglucuronic acid (UDPGA), *p*-nitrophenol, 3-aminobenzoic acid ethyl ester (MS 222) were obtained from Sigma (St Louis, MO) and 2-vinylpyridine was obtained from Aldrich (Milwaukee, WI). BSO was obtained from ICN (Montreal, Canada). All other chemicals were of analytical grade.

### *Animals*

Hatchery reared, immature rainbow trout (50–80 g) were obtained from Linwood Acres (Campellcroft, Ontario, Canada). The fish were placed in tanks supplied with dechloraminated City of Ottawa tap water and laboratory acclimated for 1 month before separating the fish randomly into 5 tanks (250 l), 15 fish per tank, supplied with identical water. Water was saturated with oxygen and maintained at 12 ± 1°C. Fish were fed 1.5% of body weight per day, but after TCB injection, feeding declined. Experiments were undertaken during the spring.

### *Exposure of Fish to TCB and Thiol Manipulation*

TCB was dissolved in corn oil heated to 60°C to obtain a stock concentration of 0.5 mg/ml. The solution was cooled to approximately 30°C and injected intraperitoneally (i.p.) at 1 mg/kg (100–160 $\mu$l) at day 0; controls received equivalent volumes of corn oil. The day following the TCB injection, fish were given daily i.p. injections of saline, GSH, BSO or lipoate in the evening. Tank water levels were lowered (to approximately 40 l) and fish were mildly anaesthesized by adding 0.05 g/l MS 222 and 0.1 g/l sodium bicarbonate to the tank. All agents were made up in physiological saline (pH 6.8), except that lipoate was dis-

solved in 1 part 6 N NaOH and 19 parts 0.2 M sodium phosphate buffer (pH 7.0). Controls and TCB-saline trout received saline injections (125–200 $\mu$l) and TCB-GSH (GSH supplemented) trout received GSH (0.25 g/kg, 125–200 $\mu$l; pH was adjusted to 6.5–6.8 by cautious addition of NaOH just before use). BSO stock was dissolved in saline with careful heating to 30°C and injection volume was 300–480 $\mu$l (6 mmol/kg). TCB-lipoate (lipoate supplemented) fish received volumes of 100–160 $\mu$l of lipoate solution (16 mg/kg).

Trout were harvested the morning following the thiol treatment for 3 days or 6 days. Fish were killed by a blow to the head and blood was collected into heparinized syringes from the caudal vessels. Whole blood and plasma were acidified with 5% 5-sulfosalicylic acid and centrifuged to deproteinize. Supernatants were stored at −20°C. Organs were quickly excised, freed from blood and snap frozen in liquid nitrogen, and stored at −80°C (<2 months) until analyzed.

### *Preparation of Tissue Samples*

Tissues were homogenized in ice-cold Tris buffer (50 mM, containing 0.25 M sucrose, and 1 mM EDTA, pH 7.5) using a Potter-Elvehjem-type glass homogenizer (liver and kidney) or a Polytron PCU-2 homogenizer (Brinkman Instruments, Rexdale, ON) (gills and white muscle). Homogenates were centrifuged at 10,000 ***g*** for 15 min at 4°C. Supernatants were stored at −80°C (<2 months) until analyzed for enzyme activities. For glutathione measurements, frozen tissue samples were homogenized in ice-cold 5% 5-sulfosalicylic acid and centrifuged at 4,000 ***g*** and 4°C for 10 min. Supernatants were collected and stored at −20°C.

### *Biochemical Analyses*

Total glutathione (TGSH) was determined according to Sen *et al.* (24) and GSSG according to Griffith (25). Enzymes were assayed at 15°C (GPX, GR, and GST) and 18°C (EROD and UDPGT) temperatures, which are within the range experienced by this species. Substrate concentrations were those of the original assay unless otherwise stated and optimal for this species. Protein content was estimated according to Lowry *et al.* (26). GPX (EC 1.11.1.9) was measured by the method of Tappel (27) with GSH substrate concentrations of 6 mM. GR (EC 1.6.4.2) was determined as described by Carlberg and Mannervick (28), and GST (EC 2.5.1.18) according to Habig *et al.* (29). EROD activity was monitored according to Burke and Mayer (30), and UDPGT activity was estimated as described by Hänninen (31).

### *RNA Slot Blot Analysis*

Total RNA was isolated from 50 mg of frozen liver tissue using a guanidinium isothiocyanate lysis, membrane binding system (RNeasy, Qiagen, Chatsworth, CA). RNA (5 $\mu$g/sample) was denatured in 50% formamide, 7% formaldehyde at 65°C for 15 min, then transferred to a nylon membrane using a vacuum slot manifold (Gibco/BRL, Burlington, Canada). In addition, an equivalent amount of total RNA from mouse liver was blotted on the same membrane as a negative hybridization control. Blots were probed with a $^{32}$P-labelled rainbow trout CYP1A cDNA probe, $_{p}$fP1-450-3′, obtained from ATCC (Rockville, MD). Probes were hybridized for 1 hr at 68°C in the presence of 100 mg/ml sonicated calf thymus DNA using a rapid hybridization system (Quickhyb, Stratagene, La Jolla, CA), and washed with 0.1 × SSC, 0.25% SDS at 62°C. Blots were exposed to X-ray film for 18 hr, stripped for 5 min with boiling 0.1 X SSC, 0.1% SDS, and then re-exposed for 24 hr to confirm stripping. Stripped blots were rehybridized with a $^{32}$P labelled oligonucleotide probe (5′-CCGACATCGCCGCT GACCCCTGGCGCCAGTTTACGTGAGCCGATC-3′) directed against the D2 region of the trout 28S RNA using the same hybridization and washing conditions as described above. Relative optical densities of the CYP1A and 28S blots were determined for each sample by densitometry scanning of the X-ray films using a Molecular Dynamics scanning laser densitometer with Imagequant software. Multiple exposures were made to ensure linear range of the film.

### *Western Blot Analysis*

Aliquots of equivalent protein content from liver homogenates of each animal were pooled for each treatment group and separated by 12% SDS-PAGE according to Laemmli (32), then transferred to nitrocellulose membranes. Blots were incubated for 2 hr with a rabbit polyclonal anti-rat CYP1A1/2 antibody (Human Biologics, Phoenix, AZ). Antibody binding was detected by chemiluminescence using a biotinylated anti-rabbit IgG followed by a streptavidin-horseradish peroxidase conjugate (ECL, Amersham, Oakville, Canada). After development, blots were exposed to Hyperfilm ECL (Amersham).

### *Statistical Analysis*

Significance of changes in means of same day groups was tested with one-way analysis of variance (ANOVA), using the software Statistix 4.1. The location of significance was determined using the Least Significant Difference method (LSD). Significant differences between 3 days and 6 days of each treatment (control, saline, GSH, BSO, and lipoate) were tested using the Student's *t*-test for unpaired sample means.

## RESULTS

Injection of TCB and treatments resulted in only one mortality in the lipoate supplemented group (6 days). Other-

wise, no fish showed any sign of distress as a result of the daily injections.

### *GSH Status*

GSH SUPPLEMENTATION. Following 3 days of GSH supplementation, GSH levels in TCB-exposed trout significantly increased in liver (3.6-fold), kidney (2.1-fold), and plasma (6.5-fold) compared with the TCB-saline treated group ($p < 0.001$) (Table 1). Consistent effects were observed in TGSH results; blood TGSH levels were 1.3-fold higher in GSH supplemented compared with TCB-saline treated fish. Tissue GSH and TGSH contents did not differ among controls, TCB-saline, and lipoate-treated fish. However, plasma GSH content was significantly higher in TCB-saline and lipoate-supplemented fish than in controls.

Following 6 days of GSH supplementation, GSH levels were significantly elevated in liver (2.5-fold), kidney (2.1-fold), gill (1.5-fold), muscle (2-fold), and plasma (8.9-fold) compared with TCB-saline injected fish. Renal and plasma GSH and TGSH levels did not differ among controls, TCB-saline, and TCB-lipoate-treated groups. However, GSH levels in liver, gill, and muscle were significantly different in TCB-saline and lipoate-supplemented trout compared with controls.

GSH DEFICIENCY. BSO treatment for 3 days resulted in multifold decreases of tissue GSH levels. Compared with TCB-saline trout, GSH concentration in GSH deficient TCB-exposed fish were decreased in liver 4.1-fold, kidney 2.8-fold, gill 1.7-fold ($p < 0.001$), muscle 5.8-fold ($p < 0.01$), and blood 1.8-fold. Following the 6 day BSO treatment, tissue GSH levels further decreased. Tissue GSH content was 1.2- to 2.1-fold lower following 6 days than 3 days of BSO treatment. White muscle was the only tissue not to show continued decreases in GSH and TGSH content following 6 days of BSO treatment.

LIPOATE SUPPLEMENTATION. Lipoate supplementation did not significantly influence tissue GSH content. TCB-lipoate supplemented fish did, however, maintain a favourable GSH redox status with lower tissue GSSG/GSH ratios than in other TCB groups and controls. GSSG/GSH ratios were particularly low in liver, kidney, muscle, blood, and plasma following 3 days and 6 days of lipoate supplementation.

TISSUE GSSG/GSH RATIO IN RESPONSE TO TCB TREATMENT. GSSG/GSH ratio in TCB-saline fish were significantly higher in liver and plasma than in all other TCB treatment groups following the 3 days ($p < 0.05$), but not the 6 days treatment with saline. GSSG/GSH ratio in liver, kidney, and gill were the lowest in GSH deficient trout compared with all other groups for the 3 days and 6 days of thiol manipulations. In muscle and blood, GSSG/GSH ratio were significantly higher in GSH-deficient than in lipoate-supplemented fish. In general, BSO and lipoate-treated fish had the lowest GSSG concentrations compared with controls, TCB-saline, and TCB-GSH treatment groups. GSSG content in GSH-supplemented trout were significantly higher in most tissues than in any of the other TCB groups or controls. Plasma GSSG levels in GSH-supplemented fish were markedly increased, resulting in a (1000*) GSSG/GSH ratio of 874–895, 6-, and 15-fold higher than in TCB-saline treated trout.

### *Glutathione Redox Cycle*

GPX activities in liver and gill of GSH-supplemented fish were elevated following 3 days of GSH treatment (Table 2). GPX activities in all tissues were significantly higher in GSH-supplemented than in GSH deficient trout ($p < 0.05$) following 3 days and 6 days of GSH manipulations. Gill GPX activity significantly increased in TCB-saline treated fish from 3 days to 6 days of saline treatment ($p < 0.01$) and was highest compared with other groups following 6 days of treatment ($p < 0.05$). However, gill GPX activity in lipoate-supplemented trout decreased from 3 days to 6 days of lipoate supplementation ($p < 0.05$).

Gill GR activities in TCB-saline and GSH deficient fish were significantly increased from 3 days to 6 days of saline and BSO treatments ($p < 0.01$). As observed for GPX activities, GR activities were also highest in GSH-rich tissues following the 3 days of GSH supplementation. GR activities in other TCB groups and controls remained for most tissues unchanged. In general, GPX and GR activities increased more rapidly in tissues of GSH supplemented fish compared with the other TCB groups, showing higher activities by 3 days of GSH supplementation. In contrast, tissue GPX and GR activities in GSH deficient fish were low compared with other TCB groups following 3 days and 6 days of BSO treatment.

### Ah *Gene Battery*

PHASE I ENZYMES. Hepatic EROD activity was 17- and 12.5-fold higher in TCB-saline trout (3 days and 6 days, respectively, $p < 0.01$) than in controls (Table 3). This induction in EROD activity was markedly potentiated in the GSH-rich livers by 2.7-fold (3 days and 6 days, $p < 0.01$ and $p < 0.05$, respectively) compared with TCB-saline fish. EROD activity in GSH-rich livers was 46- and 34-fold higher (3 days and 6 days) than in controls. This effect was fully reversed in the GSH-deficient livers as activities were 19- and 13-fold lower than in GSH-supplemented trout, but still 2.4- and 2.5-fold higher than in controls (3 days and 6 days, $p < 0.05$). TCB induced EROD activity was also lower in lipoate-supplemented fish than in TCB-saline treated trout. EROD activities were 4.1- and 7.3-fold (3 days and 6 days, $p < 0.01$ and $p < 0.05$, respectively) higher than in controls. A similar trend was observed in the kid-

**TABLE 1. Glutathione status in different tissues of TCB-treated rainbow trout**

| | Control | TCB-saline | TCB-GSH | TCB-BSO | TCB-lipoate |
|---|---|---|---|---|---|
| **Concentrations in liver, kidney and gills** | | | | | |
| Liver | | | | | |
| *3 Days* | | | | | |
| GSH | 1423 ± 176 | 1098 ± 120[1] | 3957 ± 811[#] | 270 ± 60[#] | 1091 ± 203 |
| GSSG | 65 ± 21 | 84 ± 15 | 148 ± 53[a] | 5 ± 1[#] | 47 ± 11[a] |
| TGSH | 1554 ± 201 | 1266 ± 127[1] | 4252 ± 859[#] | 280 ± 60[#] | 1186 ± 218 |
| Ratio | 44 ± 12 | 79 ± 17[#,1] | 37 ± 11 | 23 ± 7.9 | 43 ± 8 |
| *6 Days* | | | | | |
| GSH | 1136 ± 140[a] | 1715 ± 88[a] | 4214 ± 637[#] | 126 ± 36[#] | 1483 ± 105 |
| GSSG | 50 ± 10 | 67 ± 8 | 212 ± 42[#] | 3 ± 0.3[#] | 47 ± 4 |
| TGSH | 1236 ± 153[a] | 1850 ± 88[a] | 4638 ± 716[#] | 132 ± 36[#] | 1576 ± 100 |
| Ratio | 44 ± 7 | 40 ± 6 | 50 ± 4* | 31 ± 6[■] | 33 ± 4[■] |
| Kidney | | | | | |
| *3 Days* | | | | | |
| GSH | 1674 ± 147 | 1826 ± 169 | 3762 ± 344[#] | 651 ± 36[#,1] | 1728 ± 242 |
| GSSG | 85 ± 13[1] | 72 ± 10[a,1] | 111 ± 9[a] | 6 ± 0.4[#] | 32 ± 5[#] |
| TGSH | 1844 ± 156 | 1970 ± 182 | 3986 ± 357[#] | 663 ± 36[#,1] | 1792 ± 243 |
| Ratio | 52 ± 9*[,1] | 39 ± 5 | 30 ± 2[■] | 10 ± 5[#] | 20 ± 4[#] |
| *6 Days* | | | | | |
| GSH | 2051 ± 27 | 1897 ± 229 | 3942 ± 445[#] | 426 ± 9.8[#] | 1630 ± 176 |
| GSSG | 36 ± 2 | 44 ± 4[a] | 122 ± 8[#] | 6 ± 0.4[#] | 30 ± 5[a] |
| TGSH | 2122 ± 26 | 1986 ± 228 | 4185 ± 445[#] | 438 ± 10[#] | 1690 ± 182 |
| Ratio | 17 ± 9[■] | 26 ± 5[a] | 32 ± 4* | 14 ± 3[■,a] | 18 ± 2[■] |
| Gill | | | | | |
| *3 Days* | | | | | |
| GSH | 1267 ± 45[a] | 1387 ± 79 | 1677 ± 153[a] | 808 ± 57[#,1] | 1360 ± 164 |
| GSSG | 36 ± 4* | 38 ± 4*[,1] | 80 ± 9[#] | 16 ± 3[■,1] | 29 ± 5* |
| TGSH | 1339 ± 48 | 1462 ± 83 | 1836 ± 161[#] | 840 ± 60[#,1] | 1418 ± 171 |
| Ratio | 28 ± 3 | 27 ± 3 | 48 ± 5[#] | 20 ± 3 | 22 ± 2 |
| *6 Days* | | | | | |
| GSH | 1003 ± 128 | 1188 ± 62 | 1783 ± 49[#] | 501 ± 48[#] | 1445 ± 53[#] |
| GSSG | 29 ± 5 | 24 ± 3 | 66 ± 15[#] | 5 ± 2[#] | 29 ± 0.4 |
| TGSH | 1061 ± 139 | 1236 ± 67 | 1915 ± 50[#] | 512 ± 50[#] | 1502 ± 52[#] |
| Ratio | 28 ± 3 | 20 ± 2[■] | 37 ± 8* | 10 ± 4[#] | 20 ± 10[■] |
| **Concentrations in white muscle, blood and plasma** | | | | | |
| | **Control** | **TCB-saline** | **TCB-GSH** | **TCB-BSO** | **TCB-lipoate** |
| Muscle | | | | | |
| *3 Days* | | | | | |
| GSH | 74 ± 13[1] | 81 ± 33 | 97 ± 20[a] | 14 ± 4[#,1] | 40 ± 7[a,1] |
| GSSG | 2 ± 0.2* | 1 ± 0.2[■] | 7 ± 0.5[#] | 0.6 ± 0.03[■] | 1 ± 0.3[■] |
| TGSH | 78 ± 13[1] | 83 ± 33 | 111 ± 20[a] | 15 ± 4[#,1] | 42 ± 7[a,1] |
| Ratio | 31 ± 9[■] | 25 ± 7[■,a] | 78 ± 12* | 60 ± 13[a,b,1] | 29 ± 8[■,b,1] |
| *6 Days* | | | | | |
| GSH | 132 ± 7* | 81 ± 21[■] | 162 ± 22*[,a] | 39 ± 6[■,b] | 99 ± 18[a,b] |
| GSSG | 2 ± 0.5* | 2 ± 0.3* | 8 ± 1.9[#] | 0.4 ± 0.1[■] | 1 ± 0.2[■] |
| TGSH | 135 ± 6.8[a,b] | 84 ± 20[■,a] | 177 ± 24* | 40 ± 6[■,b,c] | 101 ± 18[■,c] |
| Ratio | 16 ± 4[■] | 29 ± 9[a] | 48 ± 10* | 13 ± 3[■] | 8 ± 1[■,a] |
| Blood | | | | | |
| *3 Days* | | | | | |
| GSH | 756 ± 96[■] | 820 ± 97 | 1054 ± 98* | 455 ± 54[#] | 765 ± 90[■] |
| GSSG | 279 ± 35* | 207 ± 23* | 278 ± 35* | 118 ± 19[■] | 109 ± 9[■] |
| TGSH | 1313 ± 73* | 1234 ± 132[a,b] | 1610 ± 154*[,a] | 690 ± 83[■,b] | 982 ± 84[■] |
| Ratio | 407 ± 89 | 258 ± 24 | 266 ± 23 | 262 ± 38 | 152 ± 23[#] |
| *6 Days* | | | | | |
| GSH | 803 ± 120[■] | 871 ± 170 | 1225 ± 126* | 371 ± 25[#] | 681 ± 87[■] |
| GSSG | 242 ± 16* | 169 ± 14*[,a] | 373 ± 66*[,a] | 109 ± 14[■] | 96 ± 14[■] |
| TGSH | 1287 ± 123* | 1209 ± 179[a] | 1970 ± 163[#] | 588 ± 34[■,a] | 873 ± 96[■] |
| Ratio | 331 ± 54* | 223 ± 44 | 321 ± 71* | 300 ± 45* | 146 ± 24[■] |
| Plasma | | | | | |
| *3 Days* | | | | | |
| GSH | 26 ± 2.7[■] | 42 ± 4.8* | 275 ± 38[#] | 22 ± 2.5[■,1] | 54 ± 9.1* |
| GSSG | 7 ± 1.0 | 6 ± 1.3[1] | 229 ± 30[#] | 1 ± 0.2[#] | 3 ± 1.8[#] |
| TGSH | 40 ± 4.3 | 54 ± 6.2 | 733 ± 76[#] | 24 ± 2.5[#,1] | 61 ± 12 |
| Ratio | 276 ± 28[#,1] | 148 ± 35[#,1] | 874 ± 147[#] | 44 ± 12 | 49 ± 19 |
| *6 Days* | | | | | |
| GSH | 36 ± 4.6 | 38 ± 1.9 | 339 ± 69[#] | 11 ± 1.0[#] | 41 ± 5.7 |
| GSSG | 5 ± 0.7[#] | 2 ± 0.5 | 309 ± 76[#] | 1 ± 0.2[#] | 3 ± 0.9 |
| TGSH | 47 ± 5.3 | 42 ± 2.1 | 957 ± 218[#] | 12 ± 0.9[#] | 47 ± 6.6 |
| Ratio | 152 ± 22[#] | 58 ± 12 | 895 ± 80[#] | 76 ± 23 | 62 ± 17 |

Values are means ± SEM, $n = 5$ (except TCB-BSO at 6 days; $n = 4$), and are expressed in nmol/g wet weight for all tissues, except blood and plasma are expressed in $\mu$mol/l. GSH: reduced glutathione, GSSG: oxidized glutathione, TGSH: total glutathione (GSH + 2GSSG), ratio: 1000 ∗GSSG/GSH. [#](at least) $p < 0.05$, significantly different from all other groups of the same day. *$p < 0.05$, significantly different from [■]-labeled groups of the same day; [a,b,c]$p < 0.05$: significantly different from same labeled group of the same day; [1]$p < 0.05$: significantly different from 6-day treatment.

**TABLE 2. Redox regulatory enzymes in various tissues of TCB treated rainbow trout**

| | Control | TCB-saline | TCB-GSH | TCB-BSO | TCB-lipoate |
|---|---|---|---|---|---|
| *GPX* | | | | | |
| Liver | | | | | |
| 3 Days | 104 ± 7.3[1] | 117 ± 6 | 120 ± 4* | 95 ± 6.0■ | 112 ± 11 |
| 6 Days | 70 ± 6.4■ | 104 ± 4* | 130 ± 14* | 71 ± 6.9■ | 105 ± 13* |
| Kidney | | | | | |
| 3 Days | 345 ± 14 | 289 ± 19 | 294 ± 17 | 199 ± 17# | 285 ± 34 |
| 6 Days | 305 ± 12* | 263 ± 18 | 279 ± 9 | 241 ± 20■ | 247 ± 17■ |
| Gill | | | | | |
| 3 Days | 231 ± 12■,a,c,1 | 275 ± 10■,b,1 | 382 ± 39a,b,1 | 302 ± 27■,c | 447 ± 40*,1 |
| 6 Days | 313 ± 22■ | 575 ± 45* | 499 ± 21* | 361 ± 24■ | 332 ± 22■ |
| Muscle | | | | | |
| 3 Days | 33.6 ± 3.7*,1 | 33.9 ± 2.3*,1 | 32.2 ± 1.0* | 24.3 ± 0.8■ | 25.9 ± 1.5■ |
| 6 Days | 19.2 ± 2.0■,a | 22.1 ± 1.4■ | 33.4 ± 3.3* | 27.5 ± 2.5a | 26.5 ± 0.8■ |
| *GR* | | | | | |
| Liver | | | | | |
| 3 Days | 11.0 ± 0.1 | 12.5 ± 0.7 | 12.8 ± 1.7 | 11.3 ± 0.4 | 10.8 ± 0.5 |
| 6 Days | 10.5 ± 0.7■ | 11.2 ± 0.7 | 11.5 ± 1.0 | 10.8 ± 0.6 | 13.6 ± 1.1* |
| Kidney | | | | | |
| 3 Days | 35.1 ± 2.0a | 29.2 ± 1.5■ | 39.2 ± 2.0* | 27.4 ± 1.7■,a | 29.9 ± 4.0■ |
| 6 Days | 34.8 ± 2.4 | 31.3 ± 2.3 | 32.5 ± 2.6 | 30.0 ± 1.7 | 30.9 ± 1.1 |
| Gill | | | | | |
| 3 Days | 13.7 ± 0.9■,a | 14.5 ± 1.2■,1 | 22.0 ± 4.2a | 17.4 ± 1.3■,1 | 25.3 ± 2.4* |
| 6 Days | 13.5 ± 0.8■ | 38.4 ± 0.5# | 21.4 ± 2.3* | 28.7 ± 2.0# | 22.2 ± 1.8* |
| Muscle | | | | | |
| 3 Days | 2.7 ± 0.25a,b,1 | 2.2 ± 0.23■,c | 3.1 ± 0.17*,1 | 2.0 ± 0.17■,a | 1.6 ± 0.17■,b,c,1 |
| 6 Days | 1.6 ± 0.09■,a | 2.2 ± 0.19 | 2.3 ± 0.26a | 1.7 ± 0.18■ | 2.5 ± 0.26* |

Values are means ± SEM (nmol * $min^{-1}$ * mg $protein^{-1}$), $n = 5$ (except TCB/BSO at 6 days $n = 4$). GPX, glutathione peroxidase, GR, glutathione reductase.

#$p < 0.05$, significantly different from all other groups of the same day.

*$p < 0.05$, significantly different from ■-labeled groups of the same day.

a,b,c$p < 0.05$, significantly different from same-labeled groups of the same day.

1$p < 0.05$, significantly different from 6-day treatment.

ney. Renal EROD activities were significantly higher [2.4- (3 days) and 4.8- (6 days) fold] in TCB-saline ($p < 0.01$ and $p < 0.05$, respectively), 7.6- (3 days) and 5.6- (6 days) fold in GSH ($p < 0.001$ and $p < 0.01$), 2.2- (3 days) and 2.7- (6 days) fold in lipoate-supplemented trout ($p < 0.05$) compared with controls. EROD induction did not differ between lipoate supplemented and TCB-saline treated fish after 3 days, but was significantly lower after 6 days of lipoate supplementation ($p < 0.05$) than in TCB-saline-treated trout. However, kidney EROD activity in GSH-deficient tissues was not elevated compared with controls following 3 days or 6 days of BSO treatment.

PHASE II ENZYMES. Few changes were noted in tissue GST activities after exposure to TCB and following the different thiol supplementations (Table 3). GST activities significantly increased in the kidney, gills, and muscle of TCB-saline, and in gills only of lipoate-supplemented fish from the 3 days to 6 days of saline and lipoate supplementation. GST activity in gill of TCB-saline-treated fish was significantly higher than controls following the 6 day saline treatment ($p < 0.05$).

UDPGT activities in liver and kidney were unaffected by TCB exposure (Table 3). However, following 3 days of thiol manipulation, hepatic UDPGT activities were significantly higher in GSH-supplemented-trout compared with GSH-deficient and lipoate-supplemented groups ($p < 0.05$). Following 6 days of thiol supplementation, hepatic UDPGT activities were significantly higher in GSH- and lipoate-supplemented groups than in controls and TCB-saline-treated fish ($p < 0.05$).

CYP1A EXPRESSION. TCB-induced CYP1A mRNA expression severalfold in TCB-saline and GSH-supplemented groups following 3 days and 6 days of saline and GSH treatment as indicated by an increased signal intensity on slot blots in these treatments (Fig. 1). In contrast, CYP1A mRNA levels were suppressed in GSH-deficient and lipoate-supplemented fish compared with the TCB saline group, but were still elevated as compared to controls following 3 days of thiol manipulation. 28S RNA signal intensities were significantly weaker in controls and significantly stronger in lipoate-supplemented fish ($p < 0.05$) following 3 days and 6 days than in the other TCB treatment groups (data not shown). The optical density ratio of CYP1A/28S revealed a 18- and 21-fold induction in TCB-saline and

**TABLE 3. Phase I and II enzyme activities in TCB treated rainbow trout**

| | Control | TCB-saline | TCB-GSH | TCB-BSO | TCB-lipoate |
|---|---|---|---|---|---|
| *GST* | | | | | |
| Liver | | | | | |
| 3 Days | 184 ± 9 | 201 ± 4* | 165 ± 10$^{\blacksquare}$ | 167 ± 9$^{\blacksquare}$ | 159 ± 9$^{\blacksquare}$ |
| 6 Days | 203 ± 12* | 162 ± 19$^{\blacksquare}$ | 167 ± 7 | 156 ± 13$^{\blacksquare}$ | 176 ± 3 |
| Kidney | | | | | |
| 3 Days | 201 ± 21 | 150 ± 13$^{1}$ | 197 ± 25 | 183 ± 9 | 186 ± 18 |
| 6 Days | 182 ± 15 | 205 ± 11 | 202 ± 12 | 196 ± 51 | 224 ± 10 |
| Gill | | | | | |
| 3 Days | 227 ± 21 | 181 ± 12$^{\blacksquare,1}$ | 309 ± 61* | 278 ± 31* | 213 ± 16$^{1}$ |
| 6 Days | 284 ± 63$^{\blacksquare}$ | 410 ± 23* | 313 ± 42 | 320 ± 46 | 364 ± 32 |
| Muscle | | | | | |
| 3 Days | 27.8 ± 2.4*$^{,1}$ | 25.2 ± 2.3$^{1}$ | 23.9 ± 1.2 | 22.9 ± 1.8 | 22.1 ± 1.0$^{\blacksquare}$ |
| 6 Days | 17.0 ± 1.1$^{\blacksquare}$ | 19.0 ± 1.1 | 20.8 ± 1.8 | 20.8 ± 1.8* | 20.2 ± 0.7 |
| *EROD* | | | | | |
| Liver | | | | | |
| 3 Days | 2.9 ± 0.3$^{\blacksquare}$ | 50 ± 19.6$^{\#}$ | 133 ± 41$^{\#}$ | 7.1 ± 1.5* | 12.0 ± 2.6* |
| 6 Days | 4.0 ± 0.9$^{\#,\blacksquare}$ | 50 ± 13.3* | 134 ± 44$^{\#}$ | 10.1 ± 2.3$^{\blacksquare}$ | 29.3 ± 14.2$^{\blacksquare}$ |
| Kidney | | | | | |
| 3 Days | 5.5 ± 0.8$^{\blacksquare}$ | 13.4 ± 2.2*$^{,1}$ | 41.7 ± 5.0$^{\#}$ | 4.5 ± 0.8$^{\blacksquare}$ | 12.1 ± 3.2* |
| 6 Days | 5.3 ± 0.6$^{\blacksquare,b}$ | 25.6 ± 4.1* | 29.9 ± 5.5* | 6.5 ± 0.8$^{\blacksquare,a}$ | 14.2 ± 1.8$^{\blacksquare,a,b}$ |
| *UDPGT* | | | | | |
| Liver | | | | | |
| 3 Days | 468 ± 58*$^{,1}$ | 328 ± 37 | 403 ± 83* | 219 ± 17$^{\blacksquare,1}$ | 251 ± 34$^{\blacksquare,1}$ |
| 6 Days | 238 ± 54$^{\blacksquare}$ | 256 ± 36$^{\blacksquare}$ | 395 ± 31* | 369 ± 32 | 418 ± 56* |
| Kidney | | | | | |
| 3 Days | 79 ± 3.9$^{1}$ | 93 ± 6.8*$^{,1}$ | 81 ± 7.4 | 56 ± 6.6$^{\blacksquare}$ | 80 ± 15 |
| 6 Days | 42 ± 3.0 | 50 ± 7.8 | 61 ± 6.5 | 49 ± 20 | 51 ± 7 |

Values are means ± SEM, $n = 5$ (except TCB/BSO at 6 days: $n = 4$), GST, glutathione S-transferase (nmol * min$^{-1}$ * mg protein$^{-1}$), EROD, ethoxyresorufin O-deethylase (pmol * min$^{-1}$ * mg protein$^{-1}$), UDPGT, UDPglucuronosyltransferase (pmol * min$^{-1}$ * mg protein$^{-1}$).

$^{\#}$(at least) $p < 0.05$, significantly different from all other groups of the same day.

*$p < 0.05$, significantly different from $^{\blacksquare}$-labeled groups of the same day.

$^{a,b,c}p < 0.05$, significantly different from same-labeled groups of the same day.

$^{1}p < 0.05$, significantly different from 6-day treatment.

GSH rich livers and a 5.9- and 4.7-fold induction in GSH-deficient and lipoate-supplemented livers vs. controls ($p < 0.01$) following 3 days of thiol manipulation. This CYP1A/28S RNA density ratio was significantly higher in TCB-saline and GSH-supplemented fish than in GSH-deficient and lipoate-supplemented groups ($p < 0.01$). Following 6 days of thiol manipulation, the CYP1A/28S density ratio further increased to 34- and 35-fold in TCB-saline and GSH rich livers and 18- and 22.6-fold in GSH-deficient and lipoate-supplemented groups compared with controls ($p < 0.001$). However, CYP1A/28S density ratios were significantly higher in GSH-rich than GSH-deficient livers (6 days, $p < 0.05$).

Significant TCB-induced CYP1A protein expression was revealed by Western blot analysis in TCB-saline treatment group, which was potentiated in the GSH-supplemented TCB-exposed trout following 3 days of saline or GSH supplementation (Fig. 2). CYP1A protein was only weakly expressed in GSH-deficient and lipoate-supplemented TCB exposed fish. Following 6 days of thiol manipulations, CYP1A protein expression was enhanced to a similar extent in TCB-saline and GSH-supplemented groups, and was more strongly expressed in GSH-deficient and lipoate-supplemented trout at 6 days than at 3 days of BSO and lipoate supplementation.

## DISCUSSION

### *GSH Status*

Intraperitoneally administered GSH served as an effective delivery agent of GSH to tissues of rainbow trout. Uptake of GSH is known to be catalyzed by γ-glutamyl-transpeptidase (GGT, EC 2.3.2.2), a membrane bound enzyme with cysteine as preferred amino acid acceptor. Mammalian and piscine kidney GGT activities are known to be severalfold higher than in other tissues (22,24,33) yet efforts to increase intracellular GSH concentrations by administration of exogenous GSH have met with little or no success in mammals. Repeated daily i.p. injections with GSH resulted only in significant uptake of GSH in kidney and blood of rats, while there was no effect in liver, muscle, heart, and lung (20).

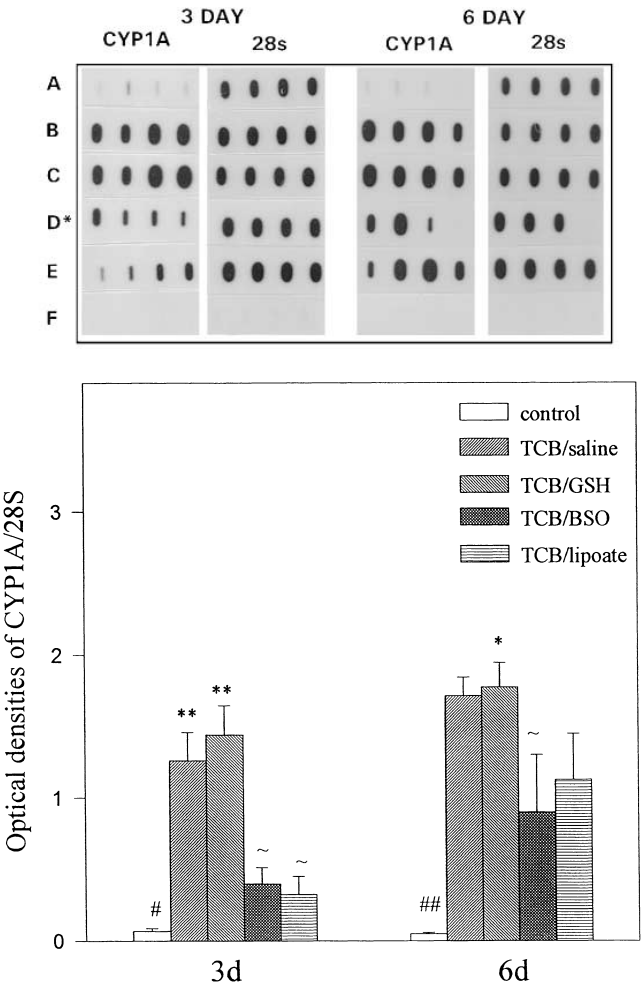


**FIG. 1. CYP1A vs 28S RNA levels. a) Five $\mu$g samples of total RNA extracted from livers of 3 day or 6 day treated animals were immobilized on nylon membrane using a vacuum slot blot apparatus. A) controls, B) TCB-saline, C) TCB-GSH, D) TCB-BSO, E) TCB-lipoate, and F) negative control RNA (5 $\mu$g from mouse liver). *: indicates $n = 3$ for TCB-BSO at 6 days. Blots were probed with a $^{32}$P-labelled CYP1A cDNA probe, and exposed to X-ray film for 18 hr. This exposure time was chosen to allow better photographic reproduction of the lower intensity signals. Blots were then stripped, re-exposed to confirm stripping, probed with a $^{32}$P-labeled oligonucleotide directed against the D2 region of the trout 28S RNA, and exposed for 6 hr. b) RNA levels were quantified by laser densitometry scanning of X-ray films to obtain CYP1A/28S ratios. Multiple exposures were made to ensure linear range of the film. CYP1A blots were exposed for 3 and 18 hr. The 3 hr exposure blot was used for denitometric determination of signal intensities, both control and induced signals were within the linear response range of the film. The 28S blots were exposed for 2, 6, and 20 hr. Two-hour exposures were used in densitometry. Error bars indicate standard error of means for each group. $^{\#}p < 0.01$, $^{\#\#}p < 0.001$, significantly different from all TCb-treatments. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from ~-labeled groups.**


**FIG. 2. Western blot of anti-rat CYP1A polyclonal antibody immunoreactive protein from trout liver. Liver homogenates of 4 animals per treatment group were pooled and 20 $\mu$g of protein per group was separated by SDS-PAGE and electroblotted to nitrocellulose membranes. Blots were probed with polyclonal anti-rat CYP1A from rabbit and detected by chemiluminescent exposure of X-ray film. A) controls, B) TCB-saline, C) TCB-GSH, D) TCB-BSO, and E) TCB-lipoate. The arrow indicates the band which co-migrates with rat CYP1A. Numbers at right indicate molecular weight standards in kDal.**

To overcome the lack of response to exogenous GSH in mammals, supplementations with N-acetylcysteine or lipoate have been used to bolster tissue GSH pools. Lipoate has been reported to increase tissue GSH levels in mice with liver concentrations enhanced by 2- to 3-fold (21). Packer *et al.* (18) has emphasized the role of lipoate as an antioxidant and its ability to protect other antioxidants, leading to increases in cellular glutathione concentrations. However, lipoate itself did not increase tissue GSH levels in TCB-treated trout in the present study, but GSSG concentrations and GSSG/GSH ratios were lower than in other groups, perhaps suggesting a sparing of GSH oxidation (Table 1). In tissues, lipoate is rapidly reduced to dihydrolipoate (18). The dihydrolipoate-lipoate couple has a lower reduction potential (−0.32 v) than the GSH-GSSG couple (−0.24 v). Thus, dihydrolipoate may directly reduce GSSG to GSH and in this way decrease the tissue GSSG/GSH ratio. BSO decreases tissue GSH content by inhibiting $\gamma$-glutamylcysteine synthetase (GCS, EC 6.3.2.2), the rate limiting enzyme of glutathione synthesis (34).

### *Effect of TCB on Antioxidant Enzymes*

Exposure of rats and fish to PCB mixtures is known to increase hepatic and muscle lipid peroxidation (15,16) A significant correlation of PCB accumulation in fish tissues with lipid peroxidation has been shown (16). We observed some effect of TCB on glutathione redox enzymes which was further influenced by the various treatments. Increased substrate availability correlated positively with GPX activities in liver, gill, and muscle of GSH-rich tissues, while GSH deficiency decreased activities of GPX in most tissues (Table 2). GSH deficiency in rats has been shown to activate hepatic and renal GR activities, but decreased GPX activity in liver by over 25% (22). The most prominent response of antioxidant enzymes in the various TCB-treatments were

noted in the gills and may be linked to the specific function of this organ in fish. Mommsen (35) reported that the metabolic activity of gills, as manifested by the high oxygen uptake, surpasses those of other tissues. Because gill metabolism contributes to excretion of hormones and possibly also toxicants (35), the activation of redox enzymes in all TCB-injected fish may imply an involvement of the gill in TCB-induced detoxication processes.

We have reported a significant activation of antioxidant enzymes in rainbow trout injected with 5 mg/kg TCB and studied 6 weeks post-injection (6). Our present results show marginally lower activation of GPX and GR in response to TCB exposure. Similarly, TCB-induced CYP1A mRNA levels were suppressed in GSH-deficient and lipoate-supplemented groups following 3 days to 6 days of thiol manipulations. It appears that several metabolic processes may be little affected immediately after TCB injection, but appear activated with time. Such a delayed response may depend on several factors, e.g., cellular thiol status (4; present data). The half-life of TCB is 7 days at a concentration of 0.8 mg/kg in this species (36) and may play a role in the activation of detoxication processes. Also, GSSG/GSH ratios were similar in most tissues of TCB-saline and control groups. GSSG/GSH ratios and GPX activities were lower in most GSH deficient tissues than in other TCB groups and controls. Thus, it appears that following drastic GSH depletion the stability of the cellular redox status is important and GPX is not activated despite exposure to TCB. GSH deficiency resulted in low GSSG content and downregulated GPX activity. This reflects a type of homeostatic adaptation that has been previously discussed by Leeuwenburgh and Ji (22).

### *TCB Effect on Ah Receptor Mediated Processes*

Exposure of rats and rat hepatocytes to TCDD and PCB congeners resulted in increased enzyme activities and expression of *Ah* battery genes (3,37). Hahn and coworkers (12,38) suggested that the AhR signal transduction pathway appeared early in vertebrate evolution by demonstrating sequence homologies between Ah receptors in mammals and fish that show highly conserved regions. Fish, like mammals, are very sensitive to TCDD and PCB congeners by induction of Ah battery enzymes (4,6). TCB exposure induced phase I (EROD activity), but not phase II enzyme activities. Whether phase II genes are induced shortly after TCB exposure and thiol manipulation is presently not clear. Xia *et al.* (37) reported that glucocorticoids have both a positive and a negative regulatory effect on several phase II genes, indicating different regulation of the various phase II genes. Such studies further emphasize the need to clarify the physiological conditions that influence the expression of these genes.

The finding that tissue thiol status modulates the gene expression of TCB-induced CYP1A is novel in xenobiotic metabolism. Thiols appear to influence CYP1A expression at different steps of the cascade. While GSH deficiency and lipoate supplementation acted by transcriptional depression of the TCB induced CYP1A gene, tissue GSH enrichment does not appear to affect transcription (Fig. 1). CYP1A protein expression in the GSH-rich livers suggests post-transcriptional or translational modifications according to the 3-day results, although the Western blot data following 6 days do not exclude the possibility of a post-translational potentiation by GSH (Fig. 2). These data again suggest a time-dependent difference in the TCB response.

In contrast to the effects of exogenous GSH is the suppression of TCB-induced CYP1A transcription and CYP1A protein expression in GSH-deficient tissues. Drew and Miners (39) demonstrated that the administration of BSO in mice did not alter CYP1A catalytic activity and pointed out its usefulness in studies focusing on the effect of GSH depletion on xenobiotic-induced toxicities. According to the results reported by Nebert and coworkers (23), GSH depletion by BSO activates *Ah* battery phase II enzymes at the pre-translational level. This activation, however, does not appear to be Ah receptor mediated, but most likely acts through the electrophilic response element (EpRE) of the *Ah* gene battery and, therefore, does not affect CYP1A (23). This group has further suggested that AhR or related proteins participate in both the TCDD/TCB inducible AhRE mediated response and the oxidative stress inducible EpRE mediated response (40). These reports, in combination with the present data, provide compelling evidence that tissue GSH status is a sensitive determinant of *Ah* battery gene expression.

We had previously hypothesized that GSH alone modifies CYP1A metabolism (41). In view of the present results, it is likely that the GSH depletion *per se* does not lead to the suppression of TCB-induced CYP1A expression. Lipoate-injected fish had similar GSH concentrations as did TCB-saline-treated individuals, yet CYP1A induction in lipoate-supplemented trout showed a similar pattern as in GSH depleted trout. Thus, the CYP1A inhibition in lipoate-supplemented trout was not dependent on low GSH content. GSSG concentrations and the GSSG/GSH ratio in most tissues of lipoate-supplemented and GSH-deficient trout were lower than those in TCB-saline and GSH-supplemented groups. GSSG in the presence of GSH has been reported to activate protein kinase C activity (42). Other studies have postulated that GSSG modulates the activation of transcription factors such as NF$\kappa$B and AP-1 (43,44). The *in vitro* binding of the Ah receptor has been shown to be regulated by redox conditions by testing Ah receptor binding under oxidant conditions and the restoration by reducing agents including dithiothreitol (14). Such observations raise the possibility of a regulatory role of oxidized thiols in cellular signal transduction pathways. Whether GSSG modulates TCB induced CYP1A transcription is yet unresolved.

Stegeman and coworkers (5,6) have described TCB as a potent CYP1A inducer at low concentrations in fish *in vivo* and *in vitro*, but TCB concentrations above 0.1 mg/kg or 0.1 $\mu$M inhibited EROD induction. This suppression of EROD activity occurred in the presence of significantly elevated CYP1A mRNA (6) and/or CYP1A protein (5). A similar inhibitory effect on CYP1A catalytic activities by PCB congeners has been reported for mammalian systems (5). Our previous study found that compounds in unbleached pulp mill effluents can lead to a similar suppression of EROD activity, although tissue content in CYP1A protein increased (45), making this inhibition of EROD activity by such environmental toxicants a relevant toxicological issue. Some tetrachlorinated dibenzofurans (TCDFs) such as 2,3,7,8-TCDF, induce scup CYP1A with good correlation of RNA, protein, and catalytic activity, whereas 2,3,6,8-TCDF lack such induction potencies at the transcriptional level (9). The structure-activity relationship of toxicants certainly determine the inducibility of CYP1A, but the present results indicate that alteration of the tissue redox status modulates the inducibility and catalytic activity of this cytochrome P450.

In conclusion, exogenous glutathione is an efficient delivery agent of GSH to tissues in rainbow trout. Active manipulations of tissue glutathione status in TCB-induced fish with either GSH, BSO, or lipoate resulted in specific biochemical responses. Activities of glutathione redox cycle enzymes were significantly higher in GSH-supplemented than in GSH-deficient tissues of TCB-exposed trout. TCB-induced CYP1A gene expression and catalytic activity were significantly altered in the different thiol manipulated systems and correlated with tissue glutathione status. This paper presents novel evidence suggesting that tissue glutathione status is a regulator of TCB induced cytochrome P4501A metabolism.

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## References

1. Asplund, L.; Svensson, B.-G.; Nillson, A.; Eriksson, U.; Jansson, B.; Jensen, S.; Wideqvist, U.; Skerfving, S. Polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (*p,p′*-DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p′*-DDE) in human plasma related to fish consumption. Arch. Environ. Health 49:477–486;1994.
2. Safe, S.H. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit. Rev. Toxicol. 24:87–149;1994.
3. Dragnev, K.H.; Nims, R.W.; Fox, S.D.; Lindahl, R.; Lubet, R.A. Relative potencies of induction of hepatic drug-metabolizing enzyme genes by individual PCB congeners. Toxicol. Appl. Pharmacol. 132:334–342;1995.
4. Otto, D.M.E.; Moon, T.W. 3,3′,4,4′-Tetrachlorobiphenyl effects on antioxidant enzymes and glutathione status in different tissues of rainbow trout. Pharmacol. Toxicol. 77:281–287; 1995.
5. Hahn, M.E.; Lamb, T.M.; Schultz, M.E.; Smolowitz, R.M.; Stegeman, J.J. Cytochrome P4501A induction and inhibition by 3,3′,4,4′-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). Aquat. Toxicol. 26:185–208;1993.
6. Gooch, J.W.; Elskus, A.A.; Kloepper-Sams, P.J.; Hahn, M.E.; Stegeman, J.J. Effects of *ortho-* and non-*ortho*-substituted polychlorinated biphenyl congeners on the hepatic monooxygnase system in scup (*Stenotomus chrysops*). Toxicol. Appl. Pharmacol. 98:422–433;1989.
7. Nebert, D.W.; Petersen, D.D.; Fornace, A.J., Jr. Cellular responses to oxidative stress: The [Ah] gene battery as a paradigm. Environ. Health Persp. 88:3–25;1990.
8. Husøy, A.-M.; Myers, M.S.; Willis, M.L.; Collier, T.K.; Celander, M.; Goksøyr, A. Immunohistochemical localization of CYP1A and CYP3A-like isozymes in hepatic and extrahepatic tissues of Atlantic cod (*Gadus morhua* L.), a marine fish. Toxicol. Appl. Pharmacol. 129:294–308;1994.
9. Hahn, M.E.; Stegeman, J.J. Regulation of cytochrome P4501A1 in teleosts: Sustained induction of CYP1A1 mRNA, protein, and catalytic activity by 2,3,7,8-tetrachlorodibenzofuran in the marine fish *Stenotomus chrysops*. Toxicol. Appl. Pharmacol. 127:187–198;1994.
10. Morrison, H.G.; Oleksiak, M.F.; Cornell, N.W.; Sogin, M.L.; Stegeman, J.J. Identification of cytochrome P-450 1A (CYP1A) genes from two teleost fish, toadfish (*Opsanus tau*) and scup (*Stenotomus chrysops*), and phylogenetic analysis of CYP1A genes. Biochem. J. 308:97–104;1995.
11. Berndtson, A.K.; Chen, T.T. Two unique CYP1 genes are expressed in response to 3-methylcholanthrene treatment in rainbow trout. Arch. Biochem. Biophys. 310:187–195;1994.
12. Hahn, M.E.; Poland, A.; Glover, E.; Stegeman, J.J. Photoaffinity labeling of the Ah receptor: Phylogenetic survey of diverse vertebrate and invertebrate species. Arch. Biochem. Biophys. 310:218–228;1994.
13. Denison, M.S.; Whitlock, J.P., Jr. Xenobiotic-inducible transcription of cytochrome P450 genes. J. Biol. Chem. 270: 18175–18178;1995.
14. Ireland, R.C.; Li, S.-Y.; Dougherty, J.J. The DNA binding of purified Ah receptor heterodimer is regulated by redox conditions. Arch. Biochem. Biophys. 319:470–480;1995.
15. Yamamoto, K.; Fukuda, N.; Shiroi, S.; Shiotsuki, Y.; Nagata, Y.; Tani, T.; Sakai, T. Ameliorative effect of dietary probucol on polychlorinated biphenyls-induced hypercholesterolemia and lipid peroxidation in the rat. Life Sciences 54:1019–1026; 1994.
16. Rudneva-Titova, I.I.; Zherko, N.V. Effects of polychlorinated biphenyls on the activity of antioxidant enzymes and lipid peroxidation in muscle and liver of two Black Sea fish species. Biochem. Moscow 59:25–31;1994.
17. Meister, A. Glutathione-ascorbic acid antioxidant system in animals. J. Biol. Chem. 269:9397–9400;1994.
18. Packer, L.; Witt, E.H.; Tritschler, H.-J. Alpha-lipoic acid as a biological antioxidant. Free Radical Biol. Med. 19:227–250; 1995.
19. Aw, T.Y. Biliary glutathione promotes the mucosal metabolism of luminal peroxidized lipids by small intestine *in vivo*. J. Clin. Invest. 94:1218–1225;1994.
20. Sen, C.K.; Atalay, M.; Hänninen, O. Exercise-induced oxidative stress: Glutathione supplementation and deficiency. J. Appl. Physiol. 77:2177–2187;1994.
21. Busse, E.; Zimmer, G.; Schopol, B.; Kornhuber, B. Influence of $\alpha$-lipoic acid on intracellular glutathione *in vitro* and *in vivo*. Arzneimittel-Forschung 42:829–831;1992.

22. Leeuwenburgh, C.; Ji, L.L. Glutathione depletion in rested and exercise mice: Biochemical consequence and adaptation. Arch. Biochem. Biophys. 316:941–949;1995.
23. Shertzer, H.G.; Vasiliou, V.; Liu, R.-M.; Tabor, M.W.; Nebert, D.W. Enzyme induction by L-buthionine (S,R)-sulfoximine in cultured mouse hepatoma cells. Chem. Res. Toxicol. 8:431–436;1995.
24. Sen, C.K.; Marin, E.; Kretschmar M.; Hänninen, O. Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. J. Appl. Physiol. 73:1265–1272;1992.
25. Griffith, O.W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106:207–212;1980.
26. Lowry, O.H.; Roseborough, A.L.; Farr A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275;1951.
27. Tappel, A.L. Glutathione peroxidase and hydroperoxides. Methods Enzymol. 52:506–513;1978.
28. Carlberg, I.; Mannervik, B. Glutathione reductase. Methods Enzymol. 113:484–490;1985.
29. Habig, W.H.; Papst, M.J.; Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 25:7130–7139;1974.
30. Burke, M.D.; Mayer, R.T. Ethoxyresorufin: Direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Disp. 2:583–588;1974.
31. Hänninen, O. On the metabolic regulation in the glucuronic acid pathway in the rat tissues. Ann. Acad. Sci. Fenn. A2 142:1–96;1968.
32. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685; 1970.
33. Otto, D.M.E.; Moon, T.W. Endogenous antioxidant systems of two teleost fish, the rainbow trout and the black bullhead, and the effect of age. Fish Physiol. Biochem. 15:349–350; 1996.
34. Meister, A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol. Ther. 51:155–194;1991.
35. Mommsen T.P. Metabolism of the fish gill. Fish Physiol. XB: 203–238;1984.
36. Gilbert, N.L.; Cloutier, M.-J.; Spear, P.A. Retinoic acid hydroxylation in rainbow trout (*Oncorhynchus mykiss*) and the effect of a coplanar PCB, 3,3′,4,4′-tetrachlorobiphenyl. Aquat. Toxicol. 32:177–187;1995.
37. Xiao, G.-H.; Pinaire, J.A.; Rodrigues, A.D.; Prough, R.A. Regulation of the *Ah* gene battery via Ah receptor-dependent and independent processes in cultured adult rat hepatocytes. Drug Metab. Disp. 23:642–650;1995.
38. Hahn, M.E.; Karchner, S.I. Evolutionary conservation of the vertebrate Ah (dioxin) receptor: Amplification and sequencing of the PAS domain of a teleost Ah receptor cDNA. Biochem. J. 310:383–387;1995.
39. Drew, R.; Miners, J.O. The effects of buthionine sulfoximine (BSO) on glutathione depletion and xenobiotic biotransformation. Biochem. Pharmacol. 33:2989–2994;1994.
40. Vasiliou, V.; Puga, A.; Chang, C.-Y.; Tabor, M.W.; Nebert, D.W. Interaction between the Ah receptor and proteins binding to the AP-1-like electrophile response element (EpRE) during murine phase II [Ah] battery gene expression. Biochem. Pharmacol. 50:2057–2068;1995.
41. Otto, D.M.E.; Sen, C.K.; Casley, W.L.; Moon, T.W. Glutathione regulates 3,3′,4,4′-tetrachlorobiphenyl induced cytochrome P450 metabolism: Evidence for a cross-talk between the two major detoxication pathways. Biochem. Mol. Biol. Int. 38:1127–1133;1996.
42. Kass, G.E.N.; Duddy, S.K.; Orrenius, S. Activation of hepatocyte protein kinase C by redox-cycling quinones. Biochem. J. 260:499–507;1989.
43. Galter D.; Mihm, S.; Dröge, W. Distinct effects of glutathione disulfide on the nuclear transcription factors κB and the activator protein-1. Eur. J. Biochem. 221:639–648;1994.
44. Sen, C.K.; Packer, L. Antioxidant and redox regulation of gene transcription. FASEB J. 10:709–720;1996.
45. Otto, D.M.E.; Lindström-Seppä, P.; Sen, C.K. Cytochrome P450 dependent enzymes and antioxidant mediated responses in rainbow trout exposed to contaminated sediments. Ecotoxicol. Environ. Saf. 27:265–280;1994a.