

Toxicokinetics and Oral Bioavailability of Halogenated Acetic Acids Mixtures in Naïve and GSTzeta-Depleted Rats

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Disinfection of drinking water typically produces a mixture of mono-, di-, and tri-halogenated acetic acids (HAAs). In this study, we investigated the toxicokinetics of HAA mixtures in naïve and glutathione transferase zeta 1 (GSTzeta)-depleted male F344 rats administered orally or iv to Mixture-1 (monobromo [MBAA]-dichloro- [DCAA], chlorodibromo- [CDBAA], tribromo- [TBAA] acetic acids) or Mixture-2 (bromochloro- [BCAA], dibromo- [DBAA], trichloro- [TCAA] bromodichloro- [BDCAA] acetic acids) at a dose of 25 $\mu\text{mol/kg}$ HAA. Serial blood samples were collected at various times up to 36 h, and the plasma concentrations of each HAA quantified by GC-ECD. Rats were pretreated for 7 d with drinking water containing 0.2 g/l DCAA to deplete the GSTzeta (GSTZ1-1) activity in the liver. An additional group of GSTzeta-depleted rats were orally dosed with each mixture and euthanized at 0.25, 0.5, 1, 2, and 4 h to determine tissue distribution of mixture components. In both mixtures, GSTzeta depletion primarily affected the toxicokinetics of di-HAAs (DCAA, BCAA, and DBAA), with the total body clearance (Cl_b) decreasing 3- to 10-fold. Interestingly, DCAA pretreatment appeared to increase the elimination of Mixture-2 tri-HAAs (TCAA and BDCAA). After oral administration, DCAA exhibited a complex time-course plasma profile with secondary peaks appearing long after completion of the initial absorption phase. This phenomenon coincided with elevated DCA levels in the lower portion of the GI tract compared to CDBAA and TBAA. Comparison of the results with previous studies employing similar or higher doses of individual HAAs indicated the primary difference in HAA toxicokinetics when administered as mixture was a reduction in Cl_b . These results suggest competitive interactions between tri- and di-HAAs beyond what would be predicted from individual HAA studies. For di-HAAs, the total dose is important, as clearance is dose dependent due to competition for GSTzeta. When considering HAA dosimetry, importance should be placed on both the components of the mixture and prior exposure history to di-HAAs.

Key Words: drinking water disinfection byproducts; halogenated acetic acids; bromo; chloro; bromochloro; chlorobromo acetic acids; oral bioavailability; toxicokinetics; pharmacokinetics; human risk assessment.

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Chlorine and bromine substituted halogenated acetic acids (HAAs) are important drinking water disinfection by-products routinely identified in surveys of municipal drinking water (Arora *et al.*, 1997; Singer *et al.*, 1995; Weinberg *et al.*, 2002). All possible combinations of chlorine and bromine in HAAs are found, although the di- and trihaloacetates tend to predominate (Pourmoghaddas *et al.*, 1993). A recent national survey reported that total HAAs burden in drinking water varies from 6 to 130 $\mu\text{g/l}$ (Weinberg *et al.*, 2002).

Trichloro- and dichloroacetic acid (TCAA, DCAA) have been the two most extensively studied HAAs due to their carcinogenic activity (Bull, 1992). Studies on the brominated and mixed bromochloro HAAs are limited. A preliminary study has found dibromo (DBAA), bromochloro (BCAA) and bromodichloroacetic acid (BDCAA) to be hepatocarcinogenic in mice (Stauber *et al.*, 1995), which is consistent with findings that bromine substitution increases mutagenicity and reproductive and developmental toxicity of HAAs (Austin *et al.*, 1996; Giller *et al.*, 1997; Hunter *et al.*, 1996; Linder *et al.*, 1994, 1997; Parrish *et al.*, 1996). Carcinogenic effects of HAAs are typically observed at high concentrations (e.g. 0.5–5 g/l) producing blood and tissue levels exceeding 750 nmol/ml (Schultz *et al.*, 2002). The high exposure levels in experimental animals compared to that identified in municipal drinking water supplies has made it difficult to estimate the health risk of individual HAAs. When results of animal studies are extrapolated downward to human exposure levels, the risk estimates can be several orders of magnitude below those calculated from epidemiology studies (Bull and Kopfler, 1991; Morris *et al.*, 1992; Murphy, 1993). Drinking water contains a mixture of HAAs whose constituents may vary due to many factors, the most important of which is the type of disinfection method used (Symanski *et al.*, 2004; Weinberg *et al.*, 2002). The chemical nature of HAAs present in finished water is significantly different between municipal sites; therefore toxicological studies of individual HAAs cannot entirely answer questions regarding the health risks of various disinfection by-products.

To increase the relevance of toxicokinetic studies of HAAs to future risk assessments, it will be important to assess the effects of HAA mixtures at lower doses than used in past studies. Determination of individual components from

a mixture of HAAs can be technically challenging due to the potential for metabolism or degradation into other HAAs (Schultz *et al.*, 1999). It is now well established that GSTzeta (GSTZ1-1) is the primary enzyme in the di-HAAs metabolism pathway (Anderson *et al.*, 1999) and may be involved in the metabolism of brominated tri-HAAs (Austin and Bull, 1997; Saghir and Schultz, 2001). An important aspect of the GSTzeta pathway in both human and rodents is its susceptibility to inactivation by exposure to DCAA and other chloro-bromo di-HAAs (Tzeng *et al.*, 2000). Our previous studies have shown that reduction in GSTzeta activity due to prior DCAA exposure profoundly reduces the clearance of chloro- and bromochloro di-HAAs (Saghir and Schultz, 2002; Schultz and Sylvester, 2001). In rodents, the reduction in clearance is caused by depletion of hepatic immunoreactive GSTzeta protein levels (Anderson *et al.*, 1999; Schultz *et al.*, 2002) as opposed to altered gene expression (Ammini *et al.*, 2004). Depletion of hepatic GSTzeta protein levels can exceed 90%, making DCAA pretreatment a convenient method for modulating di-HAA metabolism and studying the impact of GSTzeta depletion on the toxicokinetics of HAA mixtures.

This study was designed to characterize the toxicokinetics and disposition of two HAAs mixtures after intravenous (iv) and oral dosing to male Fischer 344 rats with and without GSTzeta depletion. Mixture-1 contained molar equivalent concentrations of monobromo-, dichloro-, chlorodibromo-, and tribromo- acetic acids (MBAA, DCAA, CDBAA, and TBAA) and Mixture-2 consisted of bromochloro-, dibromo-, trichloro-, and bromodichloro- acetic acids (BCAA, DBAA, TCAA, and BDCAA). The selection of mixture components was based on the desire to include both di-HAAs and tri-HAAs, which are found together in drinking water, and to avoid combining tri-HAAs with di-HAAs that may be metabolites or degradation products. For example, DCAA, BCAA, and DBAA are potential metabolites of BDCAA, CDBAA, and TBAA, respectively (Austin and Bull, 1997; Saghir and Schultz, 2001) and thus were not included in the same mixture to avoid confounding the results.

MATERIALS AND METHODS

Chemicals. All halogenated acetic acids (>99% pure as free acid) were purchased from either Fluka Chemical Corp. (Milwaukee, WI) or Supelco Inc. (Bellefonte, PA). Reagent grade methyl-*tert*-butyl ether (MTBE) was purchased from Fisher Scientific (Pittsburgh, PA). Diazomethane was prepared from *N*-methyl-*N*-nitrosoguanidine as described earlier (Saghir and Schultz, 2002). All other chemicals were of the purest grade available obtained from standard sources. Dosing solutions were prepared in saline, pH was adjusted to ~7 with NaOH, sterilized using syringe-driven filter, and stored at 4°C. Dosing solutions were analyzed intermittently using gas chromatography (GC) and found to be stable during the course of the study.

Animal care. Animal care and treatment was conducted in accordance with established Institutional Animal Care and Use Committee guidelines of the Pacific Northwest National Laboratory. Twenty male Fischer-344 rats, 8–10

weeks old (236 ± 41 g) fitted with a jugular vein cannula were obtained from Taconic Laboratories (Germantown, NY) and housed individually in polycarbonate cages with wood-chip bedding and stainless steel wire tops under standard conditions (22°C, 40–60% relative humidity, 12-h light/dark cycle) and acclimated for a minimum of 48 h prior to use in experiments. Initially, rats were provided with deionized water and Purina rat chow *ad libitum*. Deionized water was used throughout the experiments to avoid unwanted exposure to HAAs, which can be present in drinking water sources that may cause some inactivation of GSTzeta. Animals were fasted overnight prior to dosing.

Time-course plasma levels of HAAs. Naïve animals (4–6 per dose group) were dosed (iv or gavage) with Mixture-1 or Mixture-2. The dose of each HAA to the animals was 25 µmol/kg, and the volume administered was 1 ml/kg. Selection of the dose was based on the results of an earlier study (Saghir and Schultz, 2002) showing the toxicokinetics of DCAA becomes linear at doses between 10 and 40 µmol/kg, which we assumed is true for other HAAs. After the initial dosing experiment, the rats were provided with 0.2 g/l DCAA in drinking water for 7 days to deplete/inactivate GSTzeta activity to ~10% of the naïve rats (Saghir and Schultz, 2002; Schultz and Sylvester, 2001). The GSTzeta-depleted rats were then switched to non-DCAA fortified water overnight (16 h) to allow for the washout of residual DCAA from the animal. This short period allows only minimal resynthesis of GSTzeta (Saghir and Schultz, 2002). GSTzeta-depleted rats (4–6 per dose group) were then dosed (iv or gavage) with Mixture-1 or Mixture-2 as described above.

Serial blood samples (0.075–0.125 ml) were collected, and plasma was obtained, mixed with 0.2 ml of ice-cold 0.1 M sodium acetate buffer (pH 5.2), and frozen at –20°C until analyses as described earlier (Saghir and Schultz, 2002). Actual plasma volumes were determined gravimetrically using tared vials and assuming plasma density of 1.0. A typical blood sampling schedule after iv dosing was 0, 3, 6, 10, 15, 20, 30, 45 min, and variously thereafter up to 36 h depending on the mixture and pretreatment. For orally dosed animals, an additional 1-min sample was obtained. Urine was collected for 24 h on dry-ice cooled traps only from naïve rats dosed iv with Mixture-1 or Mixture-2; weighed aliquots were mixed with sodium acetate buffer and stored at –80°C until analyses.

Tissue distribution. Twenty-seven noncannulated male F344 rats (190 ± 21 g) were purchased from Charles River Laboratories (Raleigh, NC) and pretreated with DCAA as described previously to deplete GSTzeta activity. After overnight washout, rats were dosed with Mixture-1 or Mixture-2 (25 µmol/kg each HAA) via gavage and euthanized at 0.25, 0.5, 1, 2 h (both mixtures), and 4 h (Mixture-1 only) after dosing. After euthanasia, the GI tract was removed, and the contents extruded, and the stomach, small intestine (equally separated into upper and lower sections), large intestine, lung, liver, kidney, and testis procured and stored at –80°C until analysis.

Extraction of HAAs from tissues. Weighed (~200 mg) aliquots of tissues in duplicate were placed in 7-ml glass vials and an internal standard (1 µg monochloro- or fluorochloro- acetic acid) was added followed by addition of 1 ml of sodium acetate buffer. Tissues were homogenized using Omni-mixer® (Sorvall, Norwalk, CT) until completely ground. Tissue homogenates were acidified with 0.1 ml of 50% H₂SO₄ and extracted in MTBE by rigorous vortexing followed by centrifugation at 2000 rpm for 30 min. Aliquots of MTBE were analyzed for HAAs as described below.

Chemical analysis. Acetate buffer fortified samples (blood, plasma, and urine) were added with 0.2 mg internal standard (monochloro- or fluorochloro- acetic acid), acidified by adding 0.025 ml of 50% sulfuric acid, and extracted in various volumes (0.2–1.0 ml) of MTBE depending on the sampling time. The extracted free acids from blood, plasma, urine, and tissues were converted to the methyl ester by adding 0.01–0.02 ml of ethereal diazomethane as described by Schultz *et al.* (1999). Samples were then analyzed by GC-ECD (Hewlett-Packard 5890-Series II, Avondale, PA).

Kinetic analysis. The toxicokinetic analysis was similar to previous studies (Saghir and Schultz, 2002; Schultz and Sylvester, 2001; Schultz *et al.*, 1999) and is briefly summarized. Individual plasma concentration-time

profiles for each HAA were analyzed by noncompartmental methods to obtain estimates of area under the plasma concentration curve ($AUC_{0 \rightarrow \infty}$), total body clearance (Cl_b), apparent volume of distribution at steady-state (V_{ss}), and the mean residence time (MRT) using WinNonlin (Pharsight Corp., Cary, NC). The plasma elimination half-life ($t_{1/2}$) was calculated as $\beta/0.693$, with β being the slope of the terminal phase of the profiles determined by linear regression. The oral absorption rate (K_a), was estimated by fitting the plasma profiles to a one-compartment clearance-volume toxicokinetic model as described previously (Schultz *et al.*, 1999). The oral bioavailability was calculated from the ratios of the average values for $AUC_{0 \rightarrow \infty}$ for the oral and iv doses.

Statistical analysis. Significant differences between toxicokinetic parameter estimates of the naïve and GSTzeta-depleted groups for HAAs of each mixture were assessed by using Student's *t*-test. A *p* value ≤ 0.05 was considered statistically significant.

RESULTS

Toxicokinetic Profiles of Individual HAAs in Mixture-1 and Impact of GSTzeta Depletion

Intravenous administration. The plasma-concentration time profiles of DCAA, CDBAA, and TBAA are presented in Figures 1A and 1B, and summary of the toxicokinetics shown in Table 1. The primary difference in the toxicokinetics among individual HAAs and naïve and GSTzeta-depleted rats was Cl_b . Because of the consistency in the V_{ss} , differences in $t_{1/2}$ largely reflect differences in clearance. In naïve rats, DCAA was rapidly eliminated from plasma as expected, with an

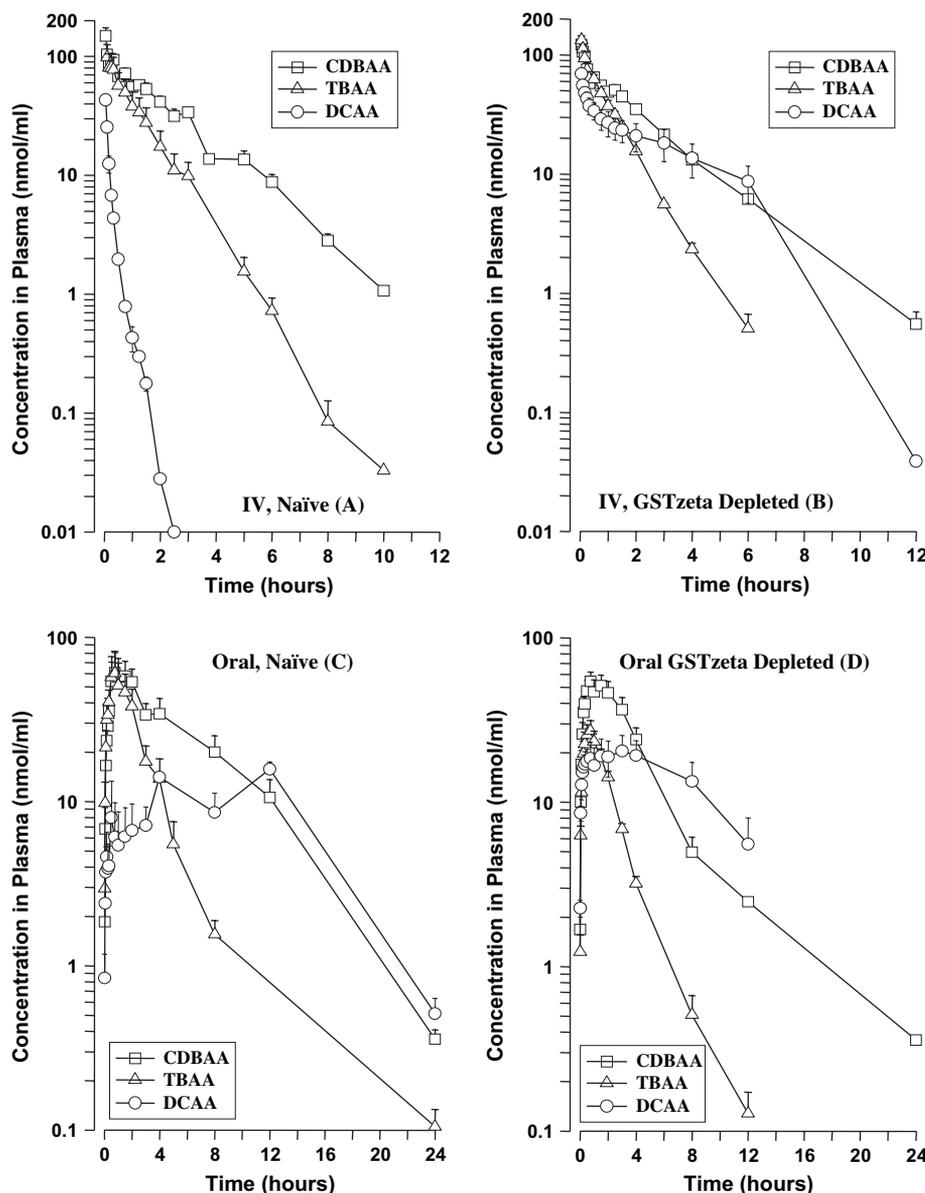


FIG. 1. Plasma concentration-time profiles of DCAA, CDBAA, and TBAA (Mixture-1) after intravenous and oral administration to naïve and GSTzeta-depleted rats (each HAA was administered at equimolar amounts of 25 mmol/kg). Not shown is MBAA, which could not be detected. The mean values \pm SE ($n = 4-6$) are shown. Error bars not shown fit within the data point.

TABLE 1
Toxicokinetic Parameters of HAAs after IV or Oral Dosing of Mixture-1 to Naïve and GSTzeta-Depleted Rats

| HA | AUC _{0→∞} (nmol h ml ⁻¹) | V _{ss} (ml kg ⁻¹) | Cl _b (ml kg ⁻¹ h ⁻¹) | MRT (h) | K _a (h ⁻¹) | t _{1/2, β} (h) | Bioavail (%) |
|---|---|--|--|--------------------------|-----------------------------------|--------------------------|-------------------|
| <i>Mixture-1, iv to naïve rats</i> | | | | | | | |
| DCAA | 8.8 ± 0.9 | 405 ± 82 | 2980 ± 332 | 0.14 ± 0.03 | — | 0.15 ± 0.04 | — |
| CDBAA | 246 ± 22 | 247 ± 25 | 105 ± 8 | 2.36 ± 0.11 | — | 1.55 ± 0.21 | — |
| TBAA | 121 ± 36 | 278 ± 51 | 291 ± 77 | 1.05 ± 0.09 | — | 0.76 ± 0.03 | — |
| <i>Mixture-1, iv to GSTzeta-depleted rats</i> | | | | | | | |
| DCAA | 145 ± 33 ^b | 668 ± 128 | 199 ± 42 ^b | 3.37 ± 0.14 ^b | — | 2.30 ± 0.29 ^b | — |
| CDBAA | 199 ± 10 | 281 ± 12 | 127 ± 6 | 2.23 ± 0.12 | — | 1.62 ± 0.13 | — |
| TBAA | 112 ± 5 | 237 ± 21 | 225 ± 9 | 1.05 ± 0.07 | — | 0.85 ± 0.11 | — |
| <i>Mixture-1, gavage to naïve rats</i> | | | | | | | |
| DCAA | 83 ± 22 | — | — | 6.55 ± 0.69 | ND | 1.37 ± 0.19 | ~900 ^a |
| CDBAA | 367 ± 74 | — | — | 5.85 ± 1.01 | 2.33 ± 0.65 | 4.59 ± 1.00 | ~149 ^a |
| TBAA | 158 ± 48 | — | — | 2.30 ± 0.24 | 3.32 ± 1.15 | 2.11 ± 0.70 | ~131 ^a |
| <i>Mixture-1, gavage to GSTzeta-depleted rats</i> | | | | | | | |
| DCAA | 206 ± 53 | — | — | 6.06 ± 0.84 | ND | 2.26 ± 0.62 | ~142 ^a |
| CDBAA | 239 ± 42 | — | — | 3.14 ± 0.17 ^c | 2.15 ± 0.28 | 1.92 ± 0.19 ^c | 97 |
| TBAA | 75 ± 8 | — | — | 3.16 ± 1.25 | 3.18 ± 0.61 | 2.19 ± 0.83 | 67 |

Note: Parameter estimates were calculated from the individual plasma concentration-time profiles of each HAA ($n = 4-6$) using noncompartmental methods with the exception of K_a , which was determined by a one-compartment clearance-volume model. The oral bioavailability was calculated from the ratio of mean values for the AUC_{0→∞}. ND = Not Determined.

^a>100 % bioavailability was associated with unusual plasma profiles for these HAAs after oral dosing. Urinary elimination of DCAA, CDBAA and TBAA over 24 h post-dosing was <0.1, 33 ± 13 and 8 ± 3% of the administered dose, respectively.

^bStatistically different from naïve animals ($p \leq 0.001$).

^cStatistically different from naïve animals ($p \leq 0.03$).

elimination $t_{1/2}$ of 9 min (Fig. 1A, Table 1). Elimination of TBAA was also rapid, with $t_{1/2}$ of 46 min, whereas CDBAA was eliminated more slowly, with a corresponding $t_{1/2}$ of 1.6 h (Fig. 1A, Table 1). Depletion of GSTzeta only affected the kinetics of DCAA in Mixture-1, with a statistically significant increase in $t_{1/2}$ (15-fold slower), AUC (16-fold increase), and reduced Cl_b (Table 1). The plasma profile of DCAA in GSTzeta-depleted rats appeared biexponential (Fig. 1B) compared to the mono-exponential decline observed in the naïve rats (Fig. 1A). Mixture-1 also contained MBAA; however, the elimination of MBAA was so rapid that in most of the samples it could not be detected even in the first sample collected 3 min after iv and 1 min after oral dosing. The percentage of the iv administered dose to naïve rats recovered in urine was <0.1% for DCAA over the 24-h post-dosing time period. Urinary recovery of CDBAA and TBAA was 33 and 8%, respectively (see footnote to Table 1).

Oral administration. All HAAs (with exception of MBAA) in Mixture-1 were rapidly absorbed after oral dosing and detected in plasma within 1 min (Figs. 1C and 1D). There was no significant difference in the K_a for CDBAA and TBAA in naïve and GSTzeta-depleted rats (Table 1). The plasma elimination of HAAs appeared to be much slower compared to that observed after iv dosing (see $t_{1/2}$ in Table 1). The plasma concentrations of CDBAA and TBAA consistently declined in either a mono- or bi-exponential manner after reaching the peak concentrations.

However, DCAA displayed a complex plasma concentration-time profile with multiple peaks appearing long after the apparent initial absorption phase. In naïve rats, the secondary plasma peaks appeared approximately 2 and 12 h after dosing. In the GSTzeta-depleted rats, the secondary peaks were less pronounced, although unusually high plasma levels persisted for an extended period of time (i.e., DCAA concentrations remained relatively unchanged from 2 to 8 h post-dosing, Fig. 1D) but were below the limit of detection 24 h after dosing. The unusual plasma profile of DCAA was exhibited by all animals (both naïve and GSTzeta-depleted) and was not limited to a few “outliers” skewing the shape of the mean time-course plasma DCAA profiles (data of individual animals not shown). This also prevented determination of K_a for DCAA. The AUC of oral doses for all three HAAs was higher than the iv route of administration, causing the calculation of oral bioavailability to be problematic, as it exceeded 100%. The exception was TBAA in the GSTzeta-depleted rats where oral bioavailability was 67% (Table 1).

Time-Course Distribution of HAAs in Mixture-1 to GI Tract of GSTzeta-Depleted Rats

Figure 2 shows the concentration-time profiles of DCAA, CDBAA, and TBAA in stomach, upper and lower small intestine and colon (GI-1, GI-2, GI-3) tissues at selected times after oral dosing. Levels of all HAAs in Mixture-1 were similar

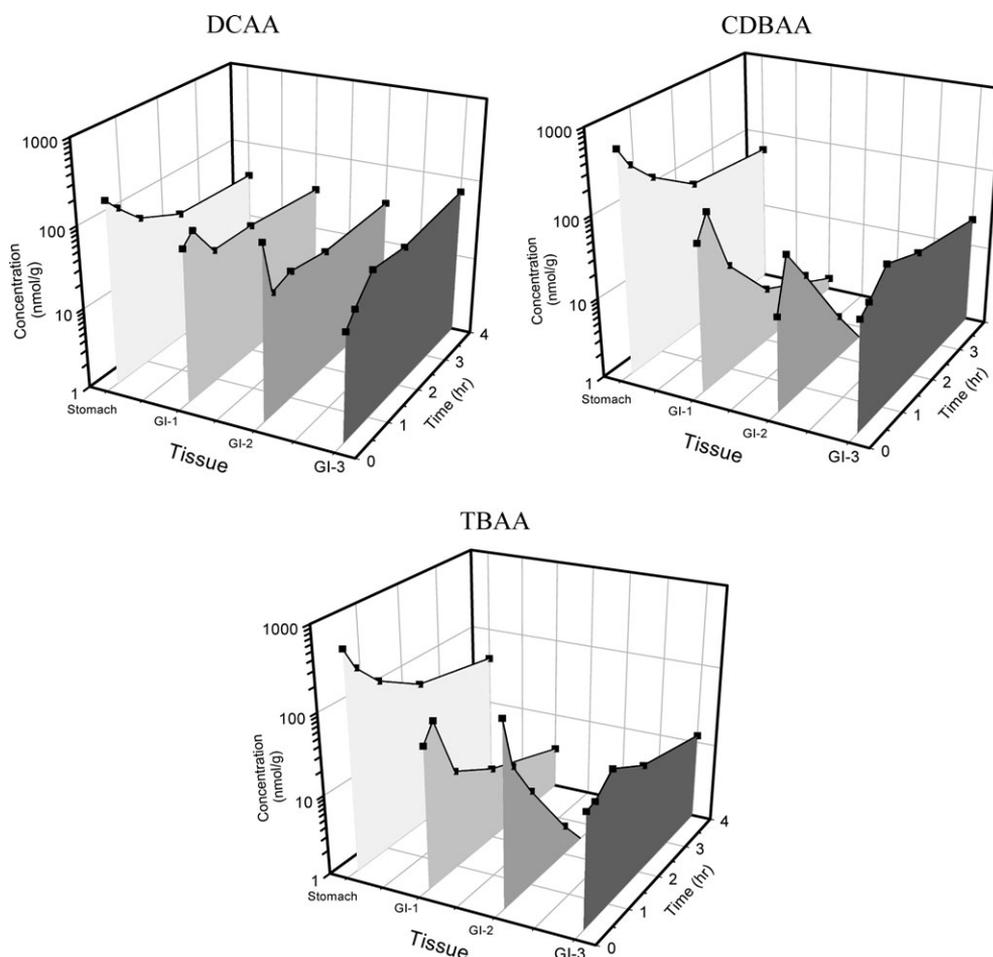


FIG. 2. Concentration-time profiles of individual HAAs of Mixture-1 in GI tract of GSTzeta-depleted rats after oral administration of 25 $\mu\text{mol/kg}$ of each HAA. Values are mean of three rats. GI-1 = upper portion of GI tract; GI-2 = mid-section of GI tract; GI-3 = large intestine.

in the stomach during the course of the study (within 4 h after dosing). However, levels of DCAA in GI-1 and GI-2 tissues were much higher than CDBAA and TBAA. Higher levels of DCAA in the upper portion of the GI tissues appears to coincide with the occurrence of secondary plasma peaks.

Toxicokinetic Profiles of Individual HAAs in Mixture-2 and Impact of GSTzeta Depletion

Intravenous administration. As noted for Mixture-1, the primary toxicokinetic difference among HAAs of Mixture-2 was in Cl_b (Table 2). The decline of all three di-HAAs, including each of the stereoisomers of BCAA, was extremely rapid both in naïve and GSTzeta-depleted rats; plasma elimination $t_{1/2}$ ranged from 4 to 11 min in naïve and was slightly longer in GSTzeta-depleted rats (11–24 min) (Figs. 3A and 3B, Table 2). The rate of plasma elimination of all three di-HAAs was so rapid that the individual profiles were difficult to discern when the tri-HAAs were plotted on the same figure, thus an additional figure was included in order to better visualize their individual profiles during the course of first 2 h after dosing (Figs. 3A and 3B

inserts). Visual inspection of the di-HAA plasma concentration-time profiles for both the naïve and GSTzeta-depleted rats indicated that they declined from plasma in a monoexponential manner. Similarly, elimination of the tri-HAAs also appeared monoexponential, albeit with a much longer plasma elimination $t_{1/2}$. Pretreatment of rats with DCAA to deplete the GSTzeta activity caused a statistically significant decrease in the plasma elimination $t_{1/2}$ of TCAA and BDCAA (see Table 2), and a slight increase in their respective Cl_b (though not statistically significant). With regard to the di-HAAs, rapid elimination after iv injection resulted in an extremely small plasma AUC, which ranged from 2–7 nmol h ml^{-1} in naïve and 7–29 nmol h ml^{-1} in GSTzeta-depleted rats (Table 2). Compared to the di-HAAs, the AUC of tri-HAAs was 50- to 190-fold higher, ranging from 291 to 1561 nmol h ml^{-1} for naïve and 306 to 1289 nmol h ml^{-1} for GSTzeta-depleted rats (Table 2). The reduced elimination of di-HAAs in GSTzeta-depleted rats caused ~ 4 -fold increase in the plasma AUC of both BCAA stereoisomers, whereas the increase was ~ 6 -fold for DBAA (Table 2). The contrast in elimination between di- and tri-HAAs was also apparent from the 2 to 3 orders of magnitude higher Cl_b of di-HAAs (Table 2).

TABLE 2
Toxicokinetic Parameters of HAAs after IV or Oral Dosing of Mixture-2 to Naïve and GSTzeta-Depleted Rats

| HA | AUC _{0→∞} (nmol h ml ⁻¹) | V _{ss} (ml ⁻¹ kg ⁻¹) | Cl _b (ml kg h ⁻¹) | MRT (h) | K _a (h ⁻¹) | t _{1/2, β} (h) | Bioavail (%) |
|---|---|--|--|-------------------------|-----------------------------------|--------------------------|------------------|
| <i>Mixture-2, iv to naïve rats</i> | | | | | | | |
| TCAA | 1561 ± 85 | 287 ± 23 | 17.1 ± 1.4 | 17.2 ± 0.9 | — | 12.03 ± 0.36 | — |
| (-)BCAA | 1.7 ± 0.1 | 680 ± 103 | 7660 ± 478 | 0.1 ± 0.01 | — | 0.06 ± 0.01 | — |
| (+)BCAA | 7.2 ± 0.6 | 393 ± 34 | 1773 ± 184 | 0.2 ± 0.01 | — | 0.19 ± 0.01 | — |
| BDCAA | 291 ± 31 | 368 ± 6 | 63.9 ± 13.0 | 4.7 ± 0.1 | — | 3.49 ± 0.14 | — |
| DBAA | 2.4 ± 0.1 | 987 ± 142 | 10540 ± 312 | 0.1 ± 0.01 | — | 0.08 ± 0.00 | — |
| <i>Mixture-2, iv to GSTzeta-depleted rats</i> | | | | | | | |
| TCAA | 1289 ± 78 | 200 ± 10 ^c | 19.7 ± 1.2 | 10.2 ± 0.2 ^b | — | 7.49 ± 0.15 ^b | — |
| (-)BCAA | 7.3 ± 1.1 ^b | 361 ± 53 ^c | 1997 ± 415 ^b | 0.2 ± 0.06 | — | 0.19 ± 0.03 ^b | — |
| (+)BCAA | 28.9 ± 3.5 ^b | 246 ± 25 ^c | 466 ± 56 ^b | 0.5 ± 0.02 ^b | — | 0.40 ± 0.02 ^b | — |
| BDCAA | 306 ± 27 | 308 ± 21 ^c | 83.9 ± 7.0 | 3.7 ± 0.1 ^b | — | 2.33 ± 0.10 ^b | — |
| DBAA | 13.2 ± 2.5 ^b | 599 ± 68 | 2390 ± 713 ^b | 0.3 ± 0.04 ^b | — | 0.22 ± 0.02 ^b | — |
| <i>Mixture-2, gavage to naïve rats</i> | | | | | | | |
| TCAA | 1247 ± 113 | — | — | 15.3 ± 1.0 | 1.55 ± 0.22 | 10.24 ± 0.85 | 82 |
| (-)BCAA | 0.9 ± 0.3 | — | — | 2.6 ± 0.7 | ND | 0.89 ± 0.21 | 52 |
| (+)BCAA | 14.3 ± 3.0 | — | — | 2.8 ± 0.3 | 5.95 ± 0.89 | 1.59 ± 0.34 | 198 ^a |
| BDCAA | 273 ± 40 | — | — | 5.4 ± 0.3 | 1.90 ± 0.55 | 3.59 ± 0.21 | 94 |
| DBAA | 6.6 ± 3.2 | — | — | 4.6 ± 1.6 | 0.57 ± 0.14 | 1.65 ± 0.56 | 275 ^a |
| <i>Mixture-2, gavage to GSTzeta-depleted rats</i> | | | | | | | |
| TCAA | 1061 ± 40 | — | — | 12.6 ± 1.1 | 1.95 ± 0.29 | 7.58 ± 0.61 | 82 |
| (-)BCAA | 11.5 ± 1.5 ^b | — | — | 2.2 ± 0.5 | 4.64 ± 0.85 | 0.71 ± 0.10 | 157 ^a |
| (+)BCAA | 30.7 ± 2.2 ^b | — | — | 2.4 ± 0.3 | 5.96 ± 1.01 | 1.18 ± 0.14 | 218 ^a |
| BDCAA | 242 ± 25 | — | — | 5.4 ± 0.3 | 2.54 ± 0.34 | 3.33 ± 0.10 | 79 |
| DBAA | 19.5 ± 1.3 ^b | — | — | 2.3 ± 0.5 | 4.70 ± 0.71 | 1.06 ± 0.22 | 148 ^a |

Note: Parameter estimates were calculated from the individual plasma concentration-time profiles of each HAA ($n = 4-6$) using noncompartmental methods with the exception of K_a , which was determined by a one-compartment clearance-volume model. The oral bioavailability was calculated from the ratio of mean values for the AUC_{0→∞}. ND = Not Determined.

^a>100% bioavailability was associated with unusual plasma profiles for these HAAs after oral dosing. Urinary elimination of TCAA, (-)BCAA, (+)BCAA, BDCAA and DBAA over 24 h post-dosing was 23 ± 2, <0.1, <0.1, 4 ± 1 and <0.1% of the administered dose, respectively.

^bStatistically different from naïve animals ($p \leq 0.01$).

^cStatistically different from naïve animals ($p \leq 0.05$).

Less than 0.1% of the administered di-HAAs were recovered in urine over 24 h after iv dosing to naïve rats. Urinary elimination of BDCAA and TCAA was 4 and 23% of the dose, respectively (see footnote to Table 2).

Oral administration. All HAAs in Mixture-2 were rapidly absorbed after oral dosing and were detected in plasma within 1 min after dosing (Figs. 3C and 3D). The decline in the plasma concentration of the tri-HAAs was similar to the pattern observed after iv dosing. Also consistent with the iv dosing results was the significantly higher rate that orally absorbed TCAA was eliminated by the GSTzeta-depleted rats (see $t_{1/2}$ values, Table 2). Elimination of (-)BCAA was much faster than (+)BCAA, similar to what was seen after iv administration. Elimination of all three di-HAAs ([-]BCAA, [+]BCAA, and DBAA) was rapid, both in the naïve and GSTzeta-depleted rats, although GSTzeta depletion resulted in a statistically significant increase in the AUC and decrease in the Cl_b of all di-HAAs (Figs. 3C and 3D, Table 2). Most other kinetic parameters remained unaffected due to GSTzeta depletion.

The elimination profiles of Mixture-2 tri-HAAs (TCAA and BDCAA) appeared monoexponential in both naïve and GSTzeta-depleted rats, consistent with the results of Mixture-1. The di-HAAs in Mixture-2 did not consistently exhibit the complex plasma concentration-time profile as was seen for DCAA, although the terminal portion of the profiles remained unusually high when compared to results from iv studies (see Fig. 3 inserts). The oral bioavailability of TCAA was 82%, both in naïve and GSTzeta-depleted rats, BDCAA bioavailability was 94% in naïve and 79% in GSTzeta-depleted rats (Table 2). With regard to the di-HAAs, estimation of oral bioavailability was problematic due to the greater AUC observed after oral dosing (Table 2).

Time-Course Tissue Distribution of HAAs in Mixture-1 and Mixture-2 in GSTzeta-Depleted Rats

Concentration of HAAs in most tissues was close to plasma levels, suggesting a rapid equilibration of HAAs between plasma and tissues (Fig. 4). There was no apparent difference between the time-course concentration pattern of CDBAA and

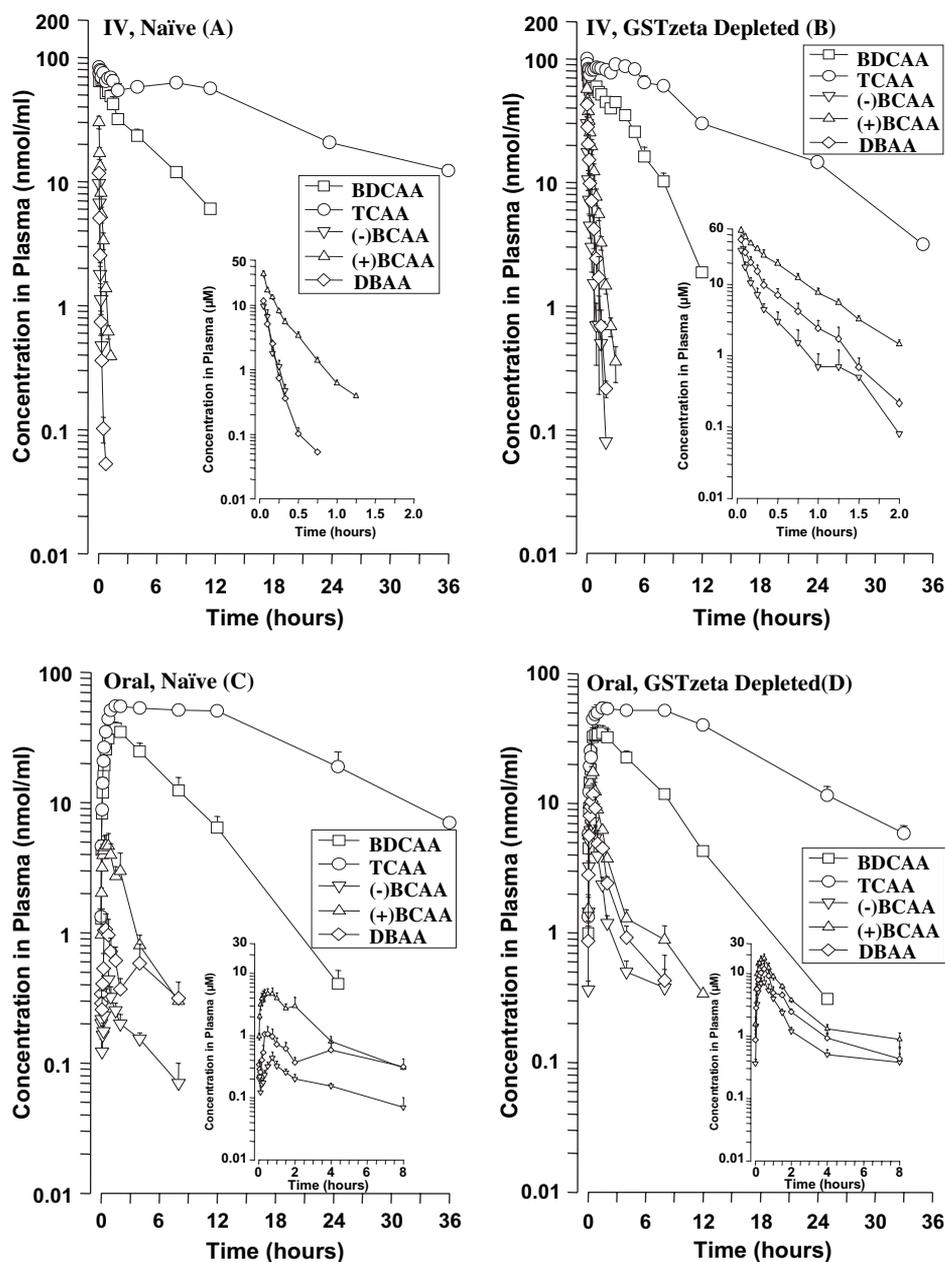


FIG. 3. Plasma concentration-time profiles of (-)BCAA, (+)BCAA, DBAA, TCAA, and BDCAA (Mixture-2) after intravenous and oral administration to naïve and GSTzeta-depleted rats (each HAA was administered at equimolar amounts of 25 $\mu\text{mol/kg}$). The mean values \pm SE ($n = 4-6$) are shown. Error bars not shown fit within the data point.

TBAA of Mixture-1 in any of the tissues examined, with the exception that CDBAA was slightly higher in lung between 1 and 2 h when compared to TBAA. The peak concentration of Mixture-1 tri-HAAs occurred between 1 and 2 h post-dosing in all tissues, coinciding with peak plasma levels. In contrast, DCAA levels were much higher than TBAA and CDBAA in the liver and testis (Fig. 4). Tissue levels of DCAA remained high and nearly constant during the later sampling times (1-4 h), which is also consistent with the complex plasma profile after oral dosing and high residual levels in the GI tract (Figs.

1C, 1D, 2, and 4). All Mixture-2 di-HAA tissue levels peaked within 1 h (Fig. 4). Tissue levels of TCAA and BDCAA were similar to the levels observed for CDBAA and TBAA and were consistent with their plasma profiles (Figs. 3D and 4).

DISCUSSION

A previous comparative toxicokinetic study of individual HAAs determined that Cl_b was the primary toxicokinetic

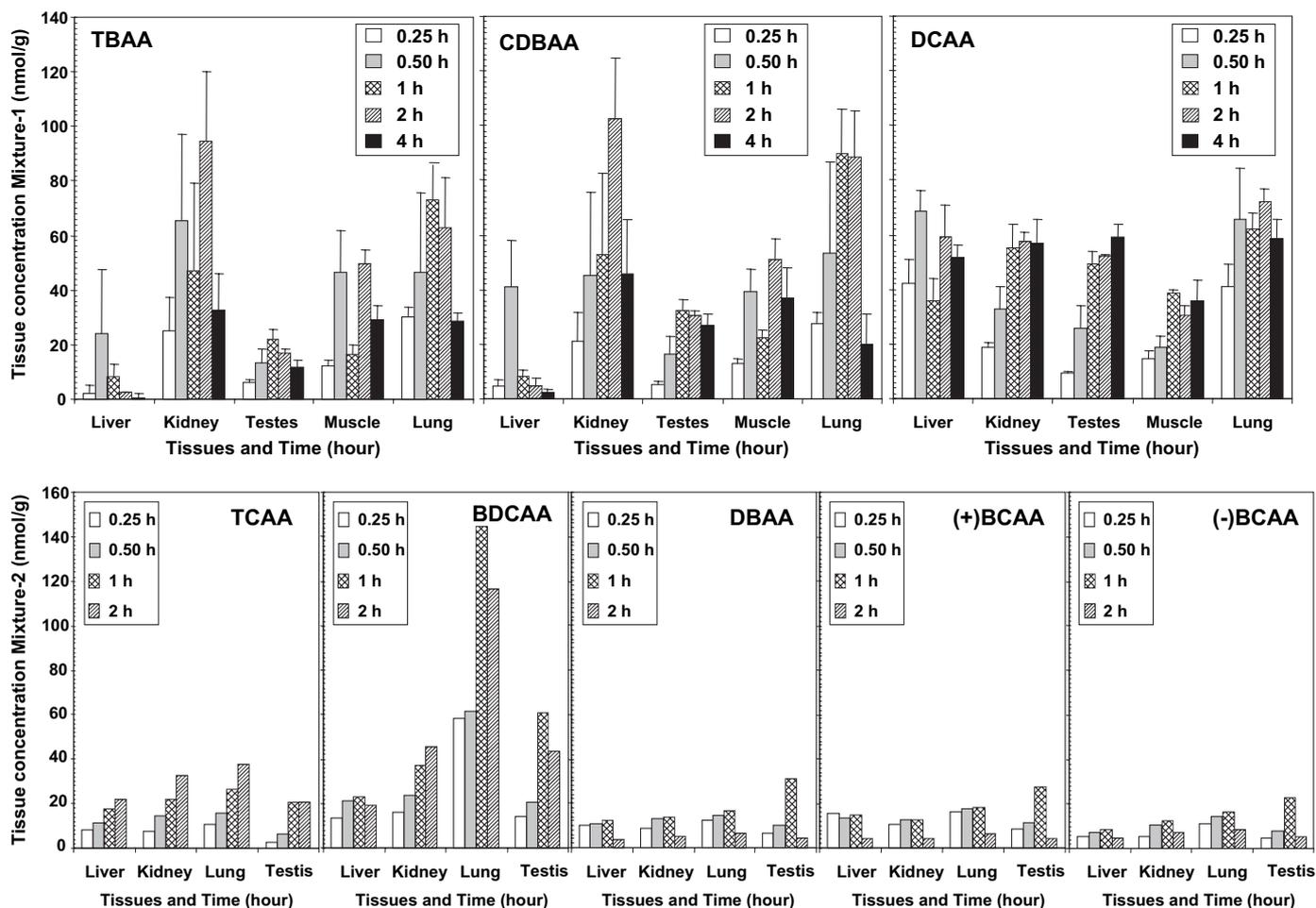


FIG. 4. Concentrations of HAAs in selected tissues of GSTzeta-depleted rats after an oral dose (25 $\mu\text{mol/kg}$) of Mixture-1 or Mixture-2 ($n = 3$ for Mixture-1, and $n = 2$ for Mixture-2).

parameter that differs among HAAs (Schultz *et al.*, 1999). Other parameters such as V_{ss} were remarkably consistent and varied little among HAAs. Differences in clearance were attributed to varying rates of renal and metabolic clearance. It was also determined that HAAs could be grouped according to number and type of halogens: di-HAAs are nearly completely eliminated by metabolism and exhibit poor renal elimination, and brominated tri-HAAs exhibit both moderate renal and metabolic elimination, while TCAA is somewhat unique in that it is poorly metabolized, with only moderate urinary elimination, which accounts for its unusually long elimination half-life. These conclusions were made from experiments using a relatively high dose of 500 $\mu\text{mol/kg}$ but appear to be consistent with results from this study. Later studies using a range of lower doses of DCAA and BDCAA have indicated Cl_b can be strongly dose dependent while other parameters are not (Merdivk *et al.*, 2000; Saghir and Schultz, 2002). For example, DCAA clearance was observed to be dose independent at doses up to 10 $\mu\text{mol/kg}$ in GSTzeta-depleted

and 40 $\mu\text{mol/kg}$ in naïve rats (Saghir and Schultz, 2002). Thus the use of a 25- $\mu\text{mol/kg}$ dose in the present study is near the dose range where HAA kinetics becomes linear, allowing assessment of mixture interactions through comparison with prior studies using similar or higher doses of individual HAAs. In the present study, our analysis of the results has tended to focus on clearance, because it was expected to be the most likely toxicokinetic parameter that may be altered from administration of HAA mixtures.

To assess whether the clearance of HAAs was altered when administered as a mixture, a comparison of values obtained from past studies of individual HAAs with those obtained in the present study is presented in Table 3. At an individual dose of 500 $\mu\text{mol/kg}$, the V_{ss} of chlorinated and brominated di- and tri-HAAs ranged from 380 to 782 ml/kg (Schultz *et al.*, 1999; Schultz and Sylvester, 2001). These values are consistent with those observed in the present study and from other studies of lower doses of selected HAAs, which are summarized in Table 3. In contrast, the Cl_b of all tri-HAAs was reduced when

TABLE 3
Comparison of Toxicokinetic Parameters of Individual HAAs Administered Intravenously as a Mixture or Individually to Naïve Rats

| Parameters | Halogenated acetic acids studied | | | | | | | |
|---|----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | TCAA | BDCAA | CDBAA | TBAA | DCAA | (-)BCAA | (+)BCAA | DBAA |
| HAAs administered as Mixture-1 (this study) dose = 25 µmol/kg | | | | | | | | |
| Cl _b ml kg ⁻¹ h ⁻¹ | — | — | 105 ± 8 | 291 ± 77 | 2980 ± 332 | — | — | — |
| V _{ss} ml kg ⁻¹ | — | — | 247 ± 25 | 278 ± 51 | 405 ± 82 | — | — | — |
| t _{1/2} h | — | — | 1.55 ± 0.21 | 0.76 ± 0.03 | 0.15 ± 0.04 | — | — | — |
| HAAs administered as Mixture-2 (this study) dose = 25 µmol/kg | | | | | | | | |
| Cl _b ml kg ⁻¹ h ⁻¹ | 17.1 ± 1.4 | 63.9 ± 13.0 | — | — | — | 7660 ± 478 | 1773 ± 184 | 10540 ± 312 |
| V _{ss} ml kg ⁻¹ | 287 ± 23 | 368 ± 6 | — | — | — | 680 ± 103 | 393 ± 34 | 987 ± 142 |
| t _{1/2} h | 12.03 ± 0.36 | 3.49 ± 0.14 | — | — | — | 0.06 ± 0.01 | 0.19 ± 0.01 | 0.08 ± 0.01 |
| Other studies^a (dose = 25 µmol/kg), each HAA administered separately to rats | | | | | | | | |
| Cl _b ml kg ⁻¹ h ⁻¹ | — | 279 ± 53.5 | 128 ± 13 | — | 5265 ± 636 | — | — | — |
| V _{ss} ml kg ⁻¹ | — | 328 ± 62 | 264 ± 45 | — | 415 ± 47 | — | — | — |
| t _{1/2} h | — | 1.3 ± 0.25 | 1.4 ± 0.25 | — | 0.08 ± 0.01 | — | — | — |
| Other studies^b (dose = 100 µmol/kg), each HAA administered separately to rats | | | | | | | | |
| Cl _b ml kg ⁻¹ h ⁻¹ | — | 138 ± 41 | — | — | 1571 ± 97 | — | — | — |
| V _{ss} ml kg ⁻¹ | — | 573 ± 179 | — | — | 223 ± 111 | — | — | — |
| t _{1/2} h | — | 3.0 ± 0.4 | — | — | 0.14 ± 0.01 | — | — | — |
| Other studies^c (dose = 500 µmol/kg), each HAA administered separately to rats | | | | | | | | |
| Cl _b ml kg ⁻¹ h ⁻¹ | 93 ± 3 | 286 ± 82 | 486 ± 153 | 754 ± 116 | 267 ± 104 | 3712 ± 140 | 1248 ± 132 | 491 ± 116 |
| V _{ss} ml kg ⁻¹ | 782 ± 117 | 730 ± 138 | 636 ± 268 | 449 ± 175 | 618 ± 318 | 380 ± 41 | 587 ± 104 | 400 ± 112 |
| t _{1/2} h | 8.0 ± 2.4 | 1.85 ± 0.30 | 1.26 ± 0.27 | 0.58 ± 0.18 | 2.40 ± 0.80 | 0.07 ± 0.01 | 0.40 ± 0.09 | 0.72 ± 0.12 |

Note. — = not part of Mixture-1 or Mixture-2, or no data is available at 25 or 100 µmol/kg dose levels when administered individually.

^aBDCAA and CDBAA are unpublished data ($n = 3-4$); DCAA data from Saghir and Schultz (2002).

^bBDCAA values are unpublished data ($n = 4$); DCAA data from Saghir and Schultz (2002).

^cData for TCAA, BDCAA, CDBAA, TBAA, DCAA, and DBAA are from Schultz *et al.* (1999); data for (-, +)BCAA are from Schultz and Sylvester (2001).

administered as a mixture compared to values obtained from individual doses ranging from 25 to 500 µmol/kg (Table 3). A similar trend appears to occur for di-HAAs, although comparisons for (-, +)BCAA and DBAA are limited to 500 µmol/kg doses, which are likely to saturate the GSTzeta metabolism pathway and lower clearance (Saghir and Schultz, 2002). The latter phenomenon was particularly pronounced for DBAA. The mixture-associated reduction in clearance for the tri-HAAs was surprising, as Cl_b would be expected to be higher at a dose of 25 µmol/kg (or the tri-HAA mixture cumulative dose of 50 µmol/kg). However, when administered as a mixture of 25 µmol/kg, the clearance of all tri-HAAs was up to 5-fold lower than when they were administered individually at doses of 25, 100, or 500 µmol/kg (Table 3), causing a corresponding increase in elimination t_{1/2}. These results are indicative of competitive elimination pathway(s) for both tri- and di-HAAs causing lower than expected dose proportionality in Cl_b of HAAs when exposed as a mixture.

Tri-HAAs are known to be metabolized by both microsomal (major) and cytosolic (minor) subcellular fractions (Austin and Bull, 1997; Merdink *et al.*, 2000). The primary metabolism of tri-HAAs occurs with dehalogenation via a cytochrome P450-

mediated process generating a di-HAA, which is subsequently metabolized by cytosolic GSTs (Austin and Bull, 1997; Merdink *et al.*, 2000; Saghir and Schultz, 2001). This was consistent with previous toxicokinetic analysis indicating that 40–70% of the total body clearance of BDCAA, CDBAA, and TBAA in rats was due to metabolic clearance (Schultz *et al.*, 1999). In the present study, levels of tri-HAAs in urine (4.3–33% dose) were lower than expected from past studies. However, it was unclear whether this was related to the mixture or the lower doses used in the present study. Interestingly, acute oral exposure to high doses of DCAA (2450 µmol/kg) is known to induce cytochrome P-450 2E1 in rats (Yang *et al.*, 1996). This would provide an explanation for the increased elimination of TCAA and BDCAA in GSTzeta-depleted rats if the DCAA pretreatment used in this study to deplete GSTzeta also stimulated microsomal metabolism.

In contrast to tri-HAA metabolism, recent studies in rats have established that chloro- and bromo- di-HAAs are primarily metabolized by GSTzeta with little or no cytochrome P450-dependent metabolism (Saghir and Schultz, 2001; Tong *et al.*, 1998). Thus, competitive elimination interactions among di-HAAs would be expected to occur through the GSTzeta and

are likely to be additive. Our results indicate some saturation of this pathway occurred at the mixture dose of 25 $\mu\text{mol/kg}$ (100 $\mu\text{mol/kg}$ total HAA dose). This would be consistent with previous findings that DCAA elimination is linear up to 40 $\mu\text{mol/kg}$ doses in naïve rats (Saghir and Schultz, 2002). Also consistent with earlier reports is the lack of significant urinary elimination of di-HAAs. In the present study, urinary elimination of all di-HAAs remained $<0.1\%$ of the dose in both of the mixtures and had no apparent effect of the presence of other di- and tri-HAAs in the mixtures.

The data presented in this study suggest that there is an interaction for the elimination of tri-HAAs when given in a mixture, and is likely due to competitive effects on metabolism. From the DCAA data, it appears that tri-HAAs can compete with di-HAAs for GSTzeta at the equimolar doses used in this study. It was unclear however, whether tri-HAAs compete with the other di-HAAs ([−, +]BCAA and DBAA) for GSTzeta, due to the lack of data for these individual di-HAAs at the equimolar doses used in this study.

With regard to oral absorption, the primary effect of the mixture appears to be enhancement of the unusual absorption pattern of HAAs, especially di-HAAs. This was most evident from the complex absorption phenomena of DCAA and the persistently high plasma levels of other di-HAAs for extended time periods (Figs. 1 and 3). This phenomenon has been reported previously, however at much higher doses (Saghir and Schultz, 2002; Schultz *et al.*, 1999). The mechanism for this phenomenon is unclear, but does not involve biliary secretion (Schultz *et al.*, 1999). Our results suggest DCAA and perhaps other di-HAAs are retained in the upper portions of the small intestine to a greater extent than tri-HAAs (Fig. 2). This may be a significant finding, as increased risk of intestinal cancer has been associated with drinking water disinfection by-products (Doyle *et al.*, 1997; Flaten, 1992). Thus, future studies of HAAs should also focus on intestinal absorption to better understand the physiological processes that regulate HAA uptake.

In conclusion, the systemic exposure of HAAs from consumption of drinking water is dynamic and affected by a number of underlying conditions including the contents of HAA mixtures and their interactions during the process of absorption from the GI tract and elimination. Results of this study when compared with previous toxicokinetic studies (Saghir and Schultz, 2002; Schultz *et al.*, 1999; Schultz and Sylvester, 2001) of individual HAAs suggest the toxicokinetics can be substantially altered when administered as a mixture. The metabolism of tri-HAAs appears to be likely affected due to competition for the enzymes responsible for their metabolism. For di-HAAs, the total dose is also important, as clearance is dose dependent, presumably due to competitive inhibition of GSTzeta. Thus, when considering HAAs dosimetry, importance should be placed on the components of the mixture (concentration of tri- and di-HAAs), total dose, and prior exposure history to di-HAAs.

SUPPLEMENTARY DATA

Supplementary data are available online at www.toxsci.oupjournals.org.

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