Use of Model-Based Compartmental Analysis to Study Effects of 2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin on Vitamin A Kinetics in Rats\(^{1,2}\)

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2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin (TCDD) is a highly toxic, widespread environmental contaminant that has dramatic adverse effects on the metabolism of vitamin A. We used model-based compartmental analysis to investigate sites and quantitative impacts of TCDD on vitamin A kinetics in rats given an oral loading dose of TCDD in oil (3.5 \(\mu\)g/kg) followed by weekly maintenance doses (0.7 \(\mu\)g/kg) or oil only. \(^{1}\)[\(^{3}\)H]Retinol in its plasma transport complex (experiment 1) or lymph containing chylomicrons labeled mainly with \(^{3}\)H[retinyl esters (experiment 2) were administered iv, and tracer kinetics in plasma, liver, carcass, urine, and feces were measured for up to 42 days. TCDD treatment caused significant reductions in liver vitamin A levels and significant changes in tracer kinetics and tracer excretion. A four-compartment model was used to fit tracer data for experiment 1; for experiment 2, compartments were added to describe the metabolism of newly absorbed vitamin A. The compartmental models predict that TCDD caused a slight delay in plasma clearance (via an increased recycling to plasma), and in liver processing, of chylomicron-derived vitamin A. Models for both experiments predict that TCDD exposure did not affect the fractional uptake of plasma retinol from the rapidly turning-over extravascular pool, but it doubled the fractional transfer of recycled retinol from slowly turning-over pools of vitamin A to plasma. The residence time for vitamin A was reduced by 70% in TCDD-treated rats, transfer into urine and feces was tripled, and vitamin A utilization rates were significantly increased. Since our results do not indicate that retinol esterification is inhibited, we hypothesize that some of the significant effects of TCDD on vitamin A metabolism result from increased catabolism and mobilization of vitamin A from slowly turning-over pools (especially the liver).

Key Words: 2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin; TCDD; rat; vitamin A; model-based compartmental analysis; kinetics.

Vitamin A\(^{4}\) is an essential nutrient that is required for normal vision, growth, reproduction, cell differentiation, embryonic development, and immune function in mammals (see review by Underwood, 1984). Much is known about the complex transport and metabolism of vitamin A (see review by Blaner and Olson, 1994). Recent research has established that retinoids are a key regulator of gene expression, exerting their biological effects by interaction with nuclear receptors (see review by Mangelsdorf et al., 1994).

Various studies have demonstrated that the normal metabolism of vitamin A is affected by alterations in vitamin A status, physiological state, or exogenous variables. One exogenous factor that has profound effects on vitamin A metabolism in many species is exposure to the persistent environmental contaminant 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) (see review by Zile, 1992). This widespread, highly toxic dioxin causes general body wasting, impaired growth and reproduction, compromised immune function, epidermal effects, and hepatotoxicity (see review by Pohjanvirta and Tuomisto, 1994). It is known that binding of TCDD to the nuclear aryl hydrocarbon (Ah) receptor affects gene transcription. In the rat, TCDD exposure causes a decrease in liver vitamin A levels, an increase in kidney vitamin A, and often an increase in plasma retinol levels (Håkansson and Ahlborg, 1985; Bank et al., 1989; Håkansson et al., 1991a, b; van Birgelen et al., 1995).

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\(^{4}\) Several compounds that show the biological activity of vitamin A are discussed here (retinol, retinyl esters, and retinoic acid).
TCDD also inhibits the normal storage of newly ingested vitamin A in liver (Håkansson and Ahlborg, 1985), most notably in perisinusoidal stellate cells (Håkansson and Hanberg, 1989), and it increases the mobilization of endogenous retinoids from liver (Håkansson et al., 1988). In addition, TCDD exposure increases excretion of vitamin A metabolites in urine and feces (Håkansson and Ahlborg, 1985; Håkansson et al., 1988), increases the glucuronidation of retinoic acid in vitro (Bank et al., 1989), and results in increased retinoic acid catabolism in microsomes from various tissues (Fiorella et al., 1995). At least some of the effects of TCDD on vitamin A homeostasis seem to be the consequence of changes in gene transcription and thus enzyme production (Zile, 1992; Pohjanvirta and Tuomisto, 1994). Furthermore, Weston et al. (1995) reported that the retinoic acid-dependent induction of genes for cellular retinoic acid-binding protein II and retinoic acid receptor-β are inhibited by TCDD.

Here we investigated the mechanisms involved in the effects of TCDD exposure on vitamin A metabolism by using model-based compartmental analysis to determine which whole-body vitamin A kinetic processes are perturbed by TCDD treatment in the rat. Although model-based compartmental analysis has not been as widely used in toxicology and pharmacology as other approaches [e.g., physiologically based pharmacokinetic modeling (PB-PK; Andersen, 1995)], it has been fruitfully applied to data on the metabolism of vitamin A and other nutrients, metabolic fuels, hormones, and organ systems (see references in Green and Green, 1990a). Here we collected data on vitamin A kinetics in plasma, tissues, and excreta of control and TCDD-treated rats and those exposed to weekly doses of TCDD after administration of a radioactive tracer. We hypothesized that TCDD might disrupt vitamin A homeostasis by affecting vitamin A utilization and recycling of retinol among plasma, liver, and extrahepatic tissues or by altering the uptake and processing of newly absorbed retinyl esters. To examine the first hypothesis, [3H]retinol tracer was administered in its normal vitamin A plasma transport complex (experiment 1) so that effects of TCDD on utilization and recycling could be determined; to address the second hypothesis, [3H]retinyl ester-labeled lymph chylomicrons were administered (experiment 2) to trace the metabolism of newly ingested vitamin A. Then we applied model-based compartmental analysis (Foster and Boston, 1983; Green and Green, 1990a) to tracer data in order to quantify effects of TCDD exposure on the kinetic behavior of vitamin A. Our results suggest that previously observed effects of TCDD on vitamin A metabolism are not related to decreases in plasma vitamin A transport to rapidly and slowly turning-over extravascular vitamin A pools, nor to defects in esterification of vitamin A, but rather to increased turnover of storage pools, resulting in net mobilization of stored vitamin A, and to increases in irreversible utilization of vitamin A.

### MATERIALS AND METHODS

#### Animals and Diets

Studies were carried out at the Karolinska Institute, Stockholm, Sweden; animal experiments were approved by the Institute's Animal Care and Use Committee. All procedures were done under light filtered through transparent films of titanium 35 (TESAB Solna AB, Solna, Sweden) to prevent photoxidation of vitamin A.

Weanling male Sprague–Dawley rats (B&K Universal, Sollentuna, Sweden) to be used as recipients of radioactive vitamin A were housed in shoebox cages in an environmentally controlled animal facility which had a 12-h light cycle (lights on 0700–1900 h). Tap water and a commercial diet (catalogue No. R34; Lactamin, Stockholm, Sweden) were provided continuously. Assuming average daily food intakes of 15–20 g/day, rats consumed 63 to 84 nmol vitamin A/day. This amount was chosen so that control rats would be in a slight positive vitamin A balance.

#### TCDD Dosing Protocol

TCDD (Lot 851:144-II) was a generous gift from Dow Chemicals (Stockholm, Sweden). A stock solution containing 100 μg TCDD/ml toluene was stored in darkness. Two working solutions of TCDD in corn oil were prepared; one, used for initial loading doses, contained 3.5 μg TCDD/ml and the second, used for subsequent weekly maintenance doses, contained 0.7 μg TCDD/ml. As discussed by Flodström et al. (1991), this dosing protocol was designed to produce a quasi steady state with respect to TCDD, but was not lethal and would presumably be effective in altering plasma and liver vitamin A levels.

After rats had been maintained on the experimental diet for 7 weeks (experiment 1) or 6 weeks (experiment 2), they were assigned by body weight to one of two groups. The loading dose (3.5 μg TCDD/kg body weight in 1 ml corn oil/kg; TCDD group) or an equal amount of corn oil (control group) was administered intragastrically. Rats to be used in long-term kinetic studies (see below) were put into metabolic cages (Techniplast Model 1700; Scanbur, Koge, Denmark); rats in short-term studies in experiment 2 were returned to shoebox cages. Maintenance doses of TCDD (0.7 μg TCDD/kg body weight in 1 ml corn oil/kg; TCDD group) or equal amounts of corn oil (control group) were given 7 days after the initial loading dose and weekly thereafter.

#### Experiment 1

**Preparation of [3H]vitamin A-labeled plasma.** [3H]Retinol-labeled plasma ([3H]retinol:retinol-binding protein (RBP)/transretin (TR)) was prepared in vivo as previously described (Green and Green, 1990b). Briefly, weanling male Sprague–Dawley rats (B&K Universal; n = 2) were fed a vitamin A-free diet for 7 weeks to deplete hepatic vitamin A stores. Then, [11,12(N)-3H]retinol (sp act = 1.4 TBq/mmol; NEN, Boston, MA; 18.5 MBq/donor) in an aqueous suspension with TWEEN 40 (Sigma Chemical Co., St. Louis, MO) was injected iv into these donor rats. After 100 min, blood was taken from the abdominal aorta into heparinized syringes. Plasma presumably containing [3H]retinol/RBP/TR (Green et al., 1985) was stored under an atmosphere of nitrogen at 4°C and used for kinetic studies over the next 2 days. Weighted replicate aliquots of the dose were taken for analysis of radioactivity (see below).

#### Kinetic study

One day before administration of retinol-labeled plasma to recipient rats, three rats in each group were killed and liver was obtained for analysis of vitamin A ("time 0"). Rats were anesthetized with CO2 and killed by extracting a large blood sample from the right cardiac ventricle. Livers were excised, bloated, weighed, frozen in liquid nitrogen, freeze-dried, and stored under an atmosphere of nitrogen at −16°C for later analysis (see below).

The kinetic study was started 2 days after administration of the first maintenance dose of TCDD. See Green and Green (1990b) for additional details on protocols used in the kinetic study. Seven rats from each experimental group (control and TCDD) were injected intragastrically with an accurately weighed amount (~0.4 g containing ~180 kBq) of [3H]retinol-labeled plasma. Over the
next 42 days, serial blood samples (n = 29; 0.1-0.25 ml) were taken from a caudal vein into microcentrifuge tubes containing 25 IU heparin; sampling times were chosen based on a geometric progression. Plasma aliquots were frozen in minivials under nitrogen for later analysis of tritium. At selected times throughout the turnover study (8 samples/rat), additional aliquots were taken for analysis of plasma retinol concentration (see below).

For each rat, urine and feces were collected as pools during day 1 (0-24 h after administration of isotope), days 1-4 (24-96 h after dosing), days 4-7 (96-168 h), and days 7-10 (168-240 h following isotope administration). At each of these times, the rat was moved to a clean metabolic cage (or, on day 10, to a shoebox cage). Urine that had accumulated in the collection cylinder was transferred into a preweighed disposable vial; the cage was rinsed twice with a total of 5-6 ml ethanol/distilled water (1:1), and rinses were combined with urine. The sample was reweighed and aliquots (3X -1 g) were weighed into minivials and frozen for later analysis of tritium (see below). Feces were transferred from their collection cylinder into sample bags, weighed, frozen, lyophilized, and stored at -16°C for later analysis of radioactivity (see below).

Forty-two days after administration of labeled plasma, rats were killed as described above. In addition to removing livers, the thyroids glands were excised and weighed (so that effects of TCDD on thyroid weight could be determined) and then returned to the carcass. Carcasses were weighed and frozen at -16°C until analyzed for radioactivity (see below).

**Experiment 2**

**Preparation of [3H]-vitamin A-labeled lymph chylomicrons.** Rats (n = 2) were anesthetized and the main mesenteric lymph duct was cannulated by the procedure of Hanberg and Trosvik (1995). On the morning after surgery, 60 TBq of [11,12(N)]Hretinol (NEN) and ~1 µg of unlabeled retinyl acetate was dissolved in corn oil (0.5 ml). Labeled oil (0.25 ml/rat) was administered orally. Lymph was collected for 6 h after dosing into tubes containing 10 µl Na2EDTA (0.4 M, pH 7.4); it was filtered through sterile gauze and diluted with unlabeled lymph. Duplicate aliquots were taken for analysis of total radioactivity and to determine the fraction of the radioactivity associated with retinol versus retinyl esters (see below). Lymph was stored at 4°C under a nitrogen atmosphere and used within 2 days of collection for in vivo studies.

**Kinetic study.** Procedures were similar to those described for experiment 1. Beginning 2 days after administration of the first TCDD maintenance dose, rats were injected iv with an accurately weighed amount of labeled lymph (~0.25 g containing -120 kBq). Rats were designated as short-term or long-term recipients. For rats in the long-term group (TCDD, n = 7; control, n = 8), serial blood samples (n = 32) were collected from a caudal vein for 42 days after dose administration. Plasma aliquots were frozen under nitrogen for later analysis of tritium and, at selected times, for retinol concentration (see below). In addition, aliquots of plasma collected during the first 2 h after dosing were frozen for analysis of tritium in retinol versus retinyl esters (see below). Urine and feces were collected as individual animal pools for 4 days after dosing; rats were then returned to shoebox cages. On day 42, long-term recipients were anesthetized and killed.

For short-term recipients in experiment 2 (3 rats/group/time), several blood samples were taken after administration of labeled lymph before rats were killed at 25 min, 8 h, and 2 days. In addition to the procedures done for experiment 1, small intestines and contents were excised, frozen in liquid nitrogen vapors, and refrozen for analysis (as below). Small intestines were examined separately from the rest of the carcass to verify that our dose was physiological (Green and Green, 1996) and to check whether TCDD caused a rapid metabolism and excretion of the administered label into bile.

**Plasma and Tissue Analyses**

Plasma samples (40-200 µl) and aliquots of the vitamin A-labeled doses were analyzed for tritium (Model 1409; Wallac Sverige AB, Upplands Väsby, Sweden) after addition of 5 ml of scintillation solution (Ecoscint A; National Diagnostics, Hillside, Stockhom, Sweden). Samples were counted twice to a final ± error of 1%. After background correction, net counts/min (cpm) were converted to disintegrations/min (dpm) using an external standard channels ratio method.

The amount of vitamin A in plasma was determined by HPLC in eight of the serial plasma samples collected from each rat in the 42-day studies. Using a modification of the procedure of Thompson et al. (1971), retinol was extracted from 50% ethanol into hexane containing 23 µM butylated hydroxytoluene (BHT); retinyl acetate was used as an internal standard. Solvent-free extracts were resuspended in ethanol and chromatographed on a Nucleosil 5 µ C18 Resolve HPLC column (150 X 4.6 mm; Phenomenex, Torrance, CA) using methanol:water (90:10 v/v, 1 mL/min) as the mobile phase. Retinol and retinyl acetate peaks were detected by UV absorbance at 328 nm (Model 486, Waters Assoc., Milford, MA). The areas under the peaks for retinol and retinyl acetate were obtained by integration (MiniChrome v1.66; Fisons Instr., VG Data Systems, Cheshire, UK) and an internal standard method was used to quantitate the amount of retinol in each sample.

Radioactivity in retinol and retinyl esters was determined in aliquots of the lymph chylomicron dose, and in plasma samples (n = 9) collected during the first 120 min after dose administration for long-term rats in experiment 2. Plasma samples were extracted as above. The hexane extracts were loaded onto columns of neutral aluminum oxide (Aldrich/RLabKemi, Stockholm, Sweden) deactivated with 5% water (Ross, 1982). Retinol esters were eluted with 3% diethyl ether in hexane and retinol with 50% diethyl ether. Fractions were analyzed for tritium (LS 1801; Beckman Instruments, Stockholm, Sweden) using Ecoscint O (National Diagnostics) as scintillation solution.

Triplicate aliquots of freeze-dried liver (0.15 g) were suspended in ethanol KOH and lipids were extracted into hexane containing 23 µM BHT (Green et al., 1985). A portion of the extract was analyzed for tritium as described for plasma, using Ecoscint O as scintillation solution. Other aliquots were analyzed for vitamin A content by HPLC as described above.

Additionally, radioactivity in liver retinol and retinyl esters was determined for short-term animals in experiment 2. Retinoids were extracted using the hexane:isopropanol:sodium sulfate method of Harms and Radin (1978) as described by Adams et al. (1995). Radioactivity in extracted retinol and retinyl esters was determined by column chromatography and liquid scintillation spectrometry as described above for plasma.

The tritium content of urine was measured (Model 1215 Rackbeta II; LKB/Wallac, Uppala, Sweden) after addition of Ecoscint O as scintillation solution. Trivalent amines were analyzed using a modification of the method of Håkansson and Ahlberg (1985). Briefly, freeze-dried feces were ground in a mortar. Quadruplicate aliquots (0.07-0.1 g) were incubated for 2 h in 4 ml methanol while mixing (200 rpm) at 56°C. After incubation, samples were vigorously vortexed for 15 min and then centrifuged for 6 min at 3000 rpm. The methanol was aspirated into glass scintillation vials and the extraction was repeated twice, using 2 ml methanol each time and 15-min incubations. After solvent had evaporated, extracts were analyzed for radioactivity using Ecoscint O as the scintillation solution.

Tritium in carcasses was determined using the method of Adams et al. (1995). Carcasses were coarsely ground using a meat grinder and five aliquots (~1.5 g each) were extracted using hexane:isopropanol:sodium sulfate. Solvent was evaporated and extracts were analyzed for radioactivity using Ecoscint O as the scintillation solution. Freeze-dried small intestines from short-term rats in experiment 2 were similarly extracted and counted.

**Kinetic Analysis**

Model-based compartmental analysis (Foster and Boston, 1983; Green and Green, 1990b) was used to develop the simplest model that fit tracer data for experiment 1. As described by Green and Green (1990b), the fraction of the dose of injected radioactivity (ffose) remaining in plasma at each sampling time for each rat was calculated as dpm/ml of plasma divided by dpm injected/estimated plasma volume, where plasma volume was approximated as mean body weight (g) during the 42-day kinetic study X 0.038 ml plasma/g.
body weight. For each rat, the fraction of the injected dose of radioactivity in liver, carcass, urine, and feces was calculated as tissue dpm divided by the total dpm injected. The fraction of the dose that was irreversibly lost by the end of each rat’s study was calculated as 1 – (dose liver + dose plasma + dose carcass) for experiment 1 and as 1 – (dose liver + dose plasma + dose carcass + dose small intestine) for experiment 2.

Individual animal data on plasma dose versus time were fit to a multieponential equation using CONSAM the conversational version 31 (Berman et al., 1983), the conversational form of the Simulation, Analysis and Modeling computer program (SAAM; Berman and Weiss, 1978). Programs were run on an IBM 80486 microcomputer. For each rat, the sum of the intercepts was used to adjust the initial estimate of plasma volume that had been calculated from body weight at the time of dose administration. Then we used CONSAM to compare normalized data for plasma, urine, feces, and irreversible loss for each rat to the three-compartment model for vitamin A metabolism proposed by Green and Green (1994). We found that a four-compartment model provided the best fit to tracer data from experiment 1 (see Results). Weighted, nonlinear regression analysis was done using CONSAM in order to determine values for the model parameters (fractional transfer coefficients \( L(IJ) \)) or the fraction of compartment J’s tracer transferred to compartment I each day) for each rat. For weighting purposes, a fractional standard deviation of 0.05 was assigned to each datum for plasma and irreversible loss, while a fractional standard deviation of 0.1 was given to urine and feces data, to reflect the potentially larger error in collection and analysis of excreta. Goodness-of-fit of the proposed model was determined by visual inspection of model-predicted versus observed data and by calculation of the estimated fractional standard deviation for each \( L(IJ) \). Parameters were considered well identified if their estimated variability was less than 0.5 (Jacquez, 1996). Next, the multiple studies feature of SAAM (Lyne et al., 1992) was used to calculate average fractional transfer coefficients for each group and the population estimate of the standard deviation.

Data from experiment 2 were handled similarly, using the final model from experiment 1 as a starting point and after addition of compartments related to chylomicron metabolism, except that estimates of plasma volume were not adjusted because of the rapid and variable removal of chylomicrons, data for urine and feces were not weighted since only one collection was made (a 4-day pool after isotope administration), and early data for fraction of dose in plasma retinyl esters and retinol were given fractional standard deviations of 0.05. A seven-compartment model with a delay element was needed to fit these data. Differences between the models presented for the two studies reflect not only the complexity needed to account for metabolism of vitamin A in chylomicrons in experiment 2, but also a difference in the number of slowly turning-over extravascular compartments needed to fit the data (one in experiment 2 versus two in experiment 1).

Using the model-predicted fractional transfer coefficients, several additional kinetic parameters were calculated for each experiment; see Green and Green (1990b) for more information on these calculations. Transit time \( T(1) \) is the length of time an average molecule of tracer spends in compartment I during a single transit. It was calculated as \( T(1) = \frac{1}{2L(IJ)} \). \( T(J) \), or mean residence time, is the total time an average retinol molecule spends in compartment I before irreversible exit from compartment I after entering the system via compartment I; it is equal to the area under the tracer response curve integrated from 0 to infinity \( \left[ AUC(LJ) \right] \). Plasma fractional catabolic rate \( FCR_p \), or the fraction of the plasma retinol pool that is irreversibly utilized each day, was calculated as \( \frac{1}{T(1)} \). The number of times that a retinol molecule recycles through compartment I before irreversible loss is the recycling number \( [nD(1)] \). It was calculated as \( \frac{[nD(1)]}{T(1)} = 1 \). \( T(SYS) \), or system mean residence time, expressed by the equation \( T(SYS) = \sum T(IJ) \), is defined as the total time a vitamin A molecule spends in the system before irreversible loss. The time that it takes for the average molecule which leaves compartment I to cycle back to compartment I is known as the recycling time \( [f(T) \). It was calculated as \( T(SYS) − T(IJ)/x(0) \).

Finally, to calculate the system disposal rate for vitamin A (DR), individual animal mean plasma retinol pool sizes (mean plasma retinol concentration measured during the kinetic study * estimated plasma volume) were multiplied by \( FCR_p \).

**RESULTS**

**Descriptive Data**

Body weights at the time of administration of vitamin A-labeled doses averaged 426 ± 34 g for rats in experiment 1 and 413 ± 35 g for those in experiment 2. Body weight gain was significantly lower for TCDD-treated versus control rats in experiment 2, and it tended to be lower in experiment 1 (Table 1). Liver weights were not significantly affected by TCDD, but relative liver weights were significantly higher in TCDD-treated rats. Thymus weights and relative thymus weights were significantly lower in TCDD-treated versus control animals in experiment 1 and tended to be lower in experiment 2.

In addition, TCDD treatment was associated with changes in vitamin A levels in plasma and liver (Table 1). Plasma retinol concentrations were higher in TCDD-treated rats, although the difference was not significant in experiment 2 (\( p < 0.066 \)). Liver vitamin A levels were reduced by 60% (experiment 1) and 44% (experiment 2) in TCDD-treated versus control rats 8 days after administration of the TCDD loading dose \( (t_0) \). Differences were even greater (97 and 95%) 42 days later \( (t_0) \). As indicated in Table 1, we estimate that control rats in both experiments were in a positive vitamin A balance, whereas TCDD-treated rats were in negative balance.

**Experiment 1: Kinetic Data**

In order to determine which aspects of whole-body vitamin A kinetics were affected by TCDD exposure, we administered \([\beta^3\text{H}]\text{retinol} \) in its physiological plasma transport complex to control and TCDD-treated rats and used model-based compartmental analysis to quantify vitamin A disposal rate and retinol recycling. Disappearance of injected \([\beta^3\text{H}]\text{retinol/RBP/TTR} \) from plasma versus time is shown as a semi-log plot in Fig. 1 for one representative control and one TCDD-treated rat. Curves for other rats were very similar. As in earlier experiments (Green and Green, 1994), the plasma pool of retinol acted kinetically as a single compartment and, in control rats, about 50% of the injected radioactivity was rapidly removed from plasma during the first 50–60 min after administration of label (Fig. 1, bottom). The early bend (1–2 h) in the curve indicates recycling of labeled retinol to plasma, primarily from a rapidly turning-over extravascular compartment. Between days 2 and 10 (Fig. 1, top), there was a slow, gradual bend in the plasma decay curve, characteristic of exchange of label
between plasma and slowly turning-over extravascular pools of vitamin A; the shape and timing of the bend is related to liver vitamin A levels (Green et al., 1987). After 10–14 days, the curve reached a constant, terminal slope. For TCDD-treated rats (Fig. 1, dotted line), the same general features in plasma tracer response curves were apparent but responses for the two groups diverged as early as 21 min after administration of the labeled dose. The earlier bend indicates a more rapid recycling of labeled retinol into plasma from the slowly turning-over extravascular pools of vitamin A in TCDD-treated rats. The steeper terminal slope indicates a higher fractional rate of loss of labeled vitamin A from the system in TCDD-treated rats compared to controls.

At 42 days after dose administration, TCDD treatment was associated with a dramatic reduction in recovery of the label in liver (1.6% versus 30% in control rats), whereas the fraction of the dose in carcass was not affected by TCDD treatment (Table 2). In both control and TCDD-treated rats, substantially more radioactivity was recovered in methanol-soluble extracts of feces than urine (Table 2). Cumulative excretion of radioactivity in urine and feces was higher in TCDD-treated versus control rats so that, at 10 days, cumulative urinary recovery was 100% higher in TCDD-treated rats and that in feces was 28% higher.

**Experiment 1: Model Development and Kinetic Parameters**

In view of some similarities in experimental design, liver vitamin A levels, and the geometry of the plasma tracer response profiles, we began model development using the three-compartment model proposed by Green and Green (1994). In that model, dietary vitamin A enters the system via a central plasma compartment; this is also the site of introduction of tracer. Plasma retinol in compartment 1 could then exchange with vitamin A in both a small, rapidly turning-over extravascular pool and a larger, slowly turning-over extravascular pool before leaving the system from the more slowly turning-over pool. The rapidly turning-over pool was postulated to represent a pool of intracellular retinol and retinol in interstitial fluid and possibly vitamin A filtered by the kidney which is in the process of being reabsorbed. The more slowly turning-over compartment would include vitamin A in retinyl ester-containing storage pools, primarily in the liver.

Plasma tracer data for individual rats in experiment 1 were compared to the three-compartment starting model. Even with the addition of separate outputs to accommodate data for urine and feces, that model did not adequately fit the current data for plasma tracer between days 2 and 10. Specifically, simulations of the three-compartment model predicted a much lower recycling of the label into plasma from the slowly turning-over pool than was observed here. Thus, we added a fourth compartment exchanging with the more slowly turning-over extravascular compartment (Fig. 2), hypothesizing that this compartment includes the least dynamic pools of body vitamin A (e.g., retinyl esters in lipid droplets of hepatic perisinusoidal stellate cells and in other large, slowly turning-over extrahepatic vitamin A stores). Addition of a fourth compartment resulted in a significant improvement in the sum of squares as determined by an F statistic (Landaw and DiStefano, 1984); thus, this structure was accepted as our working model.

Values for the model parameters [i.e., the fractional transfer
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**FIG. 1.** Tracer response profiles (fraction of injected $[^3]$H]retinol-labeled plasma remaining versus time after dose administration) in one representative control and one representative TCDD-treated rat in experiment 1. Top shows observed data (symbols) and model-predicted values (lines) for plasma fraction of dose over 42 days (control, •; TCDD, △) and for irreversible loss [1 — (fraction of dose in plasma + liver + carcass)] on day 42 (control, ○; TCDD, ▼). The bottom shows the first day's plasma data for the same rats on an expanded scale. The model-predicted lines are the responses predicted by the compartmental model shown in Fig. 2.

**TABLE 2**

Recovery of Administered Radioactivity (Fraction of Dose) in Liver, Carcass, and Excreta of Control and TCDD-Treated Rats in Experiment 1

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.300 ± 0.038</td>
<td>0.016 ± 0.039*</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.139 ± 0.049</td>
<td>0.151 ± 0.022</td>
</tr>
<tr>
<td>Cumulative urine (day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.009 ± 0.004</td>
<td>0.019 ± 0.006*</td>
</tr>
<tr>
<td>4</td>
<td>0.023 ± 0.005</td>
<td>0.048 ± 0.011*</td>
</tr>
<tr>
<td>7</td>
<td>0.031 ± 0.007</td>
<td>0.062 ± 0.014*</td>
</tr>
<tr>
<td>10</td>
<td>0.037 ± 0.009</td>
<td>0.075 ± 0.017*</td>
</tr>
<tr>
<td>Cumulative feces (day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.024 ± 0.009</td>
<td>0.036 ± 0.018</td>
</tr>
<tr>
<td>4</td>
<td>0.140 ± 0.019</td>
<td>0.185 ± 0.029</td>
</tr>
<tr>
<td>7</td>
<td>0.194 ± 0.027</td>
<td>0.258 ± 0.039*</td>
</tr>
<tr>
<td>10</td>
<td>0.235 ± 0.036</td>
<td>0.300 ± 0.046*</td>
</tr>
</tbody>
</table>

Note. Values are means ± SD (n = 7) for fraction of administered radioactivity recovered in liver and carcass at the end of the kinetic study (42 days after administration of $[^3]$H]retinol-labeled plasma to control or TCDD-treated rats and in pools of urine and feces collected on days 1, 4, 7, or 10 after tracer administration.

* Significantly different from controls (p < 0.05).

plasma from this pool [L(1,2)] was slightly but not significantly higher in the same group. The most dramatic effects of TCDD were seen on the more slowly turning-over vitamin A compartments (compartments 3 and 4): fractional release of

coefficients or L(I,J)s for each rat were adjusted to obtain the best fit of the data to the proposed model. The model's ability to fit the data is indicated by comparing the model-predicted values for plasma tracer response versus time (solid and dotted lines; Fig. 1) to the observed values (symbols). A good match was also obtained between observed and predicted data for irreversible loss on day 42 (Fig. 1) and for tracer excretion in urine and feces (data not shown). All model parameters were well identified, based on the criterion that the estimated fractional standard deviations for each L(I,J) in each rat's model was less than 0.5 and in most cases, they were less than 0.15.

Using the multiple studies feature of CONSAM (see Materials and Methods), group mean fractional transfer coefficients [L(I,J)s] and their statistical uncertainties were calculated (Table 3). L(2,1), the fractional transfer of retinol from plasma to the rapidly turning-over extravascular compartment, was significantly higher (43%) in TCDD-treated rats; the transfer to

**FIG. 2.** Working compartmental model developed to fit tracer data for plasma, carcass, irreversible loss [1 — (fraction of dose in plasma + liver + carcass)], and excreta (urine and feces) of control and TCDD-treated rats injected with $[^3]$H]retinol-labeled plasma in experiment 1. Circles represent compartments and interconnectivities are the adjustable model parameters (fractional transfer coefficients [L(I,J)s] or the fraction of compartment J's tracer transferred to compartment I each day). Compartment 1 represents plasma retinol and compartments 2–4 are extravascular pools of vitamin A; compartment 31 is the site of urinary output of tracer and compartment 32 is feces. The asterisk indicates the site of tracer introduction and U(1) represents dietary input into the system. By adjusting the values for the L(I,J)s for each individual rat, this model structure provided a good fit to the data for the 7 controls and 7 TCDD-treated rats. As an example, the differential equation used to describe the change in labeled vitamin A present in compartment 1 is $\frac{dQ_1}{dt} = L(1,2)^*Q(2) + L(1,3)^*Q(3) - [L(3,1)^*Q(1)]$, where Q, equals the amount of labeled vitamin A in compartment I and L(I,J)s are fractional transfer coefficients.
A disposal rate (DR) was significantly higher (27%) in TCDD-treated rats. The vitamin contradiction is due to the relative contributions of early and later responses to the area under the curve and the increased not significantly affected by TCDD (Table 4). This seeming took one-third the time to recycle to plasma \( [H(L)] \) of the time rats. After TCDD exposure, the average vitamin A molecule irreversibly utilized \([u(I)]\).! Although the plasma tracer response curves for a representative control and TCDD-treated rats versus time after administration of labeled chylomicrons are presented in Fig. 3. Curves for other animals followed similar patterns. The majority (90.5%) of the radioactivity in the administered lymph chylomicron dose was present as retinyl esters (Fig. 3, bottom). In control animals, essentially all of the injected radioactivity was cleared from the plasma within the first 30 min (Fig. 3, middle, solid line), reflecting lipolysis of chylomicron triglycerides and subsequent clearance of chylomicron remnants containing \([H]\)retinyl esters primarily by liver. Then, plasma radioactivity rose from \(30\) min to a peak at \(140\) min, corresponding to liver secretion of \([H]\)retinol/RBP/TTR into plasma. Subtle differ-

### Experiment 2: Kinetic Data

In order to examine effects of TCDD exposure on the metabolism of dietary vitamin A, rats in experiment 2 were injected with \([H]\)retinol-labeled lymph chylomicrons. Plasma tracer response curves for a representative control and TCDD-treated rat after administration of labeled chylomicrons are presented in Fig. 3. Curves for other animals followed similar patterns. The majority (90.5%) of the radioactivity in the administered lymph chylomicron dose was present as retinyl esters (Fig. 3, bottom). In control animals, essentially all of the injected radioactivity was cleared from the plasma within the first 30 min (Fig. 3, middle, solid line), reflecting lipolysis of chylomicron triglycerides and subsequent clearance of chylomicron remnants containing \([H]\)retinyl esters primarily by liver. Then, plasma radioactivity rose from \(30\) min to a peak at \(140\) min, corresponding to liver secretion of \([H]\)retinol/RBP/TTR into plasma. Subtle differ-

### TABLE 3

Model-Predicted Fractional Transfer Coefficients \([L(I,J)]\) for the Proposed Model of Vitamin A Metabolism in Control and TCDD-Treated Rats in Experiment 1

<table>
<thead>
<tr>
<th>(L(I,J)) (\text{(day}^{-1}))</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L(2,1))</td>
<td>13.9 ± 3.9</td>
<td>19.6 ± 3.9*</td>
</tr>
<tr>
<td>(L(1,2))</td>
<td>14.6 ± 4.8</td>
<td>18.2 ± 5.3</td>
</tr>
<tr>
<td>(L(3,1))</td>
<td>4.73 ± 1.36</td>
<td>4.03 ± 0.62</td>
</tr>
<tr>
<td>(L(1,3))</td>
<td>0.0985 ± 0.0366</td>
<td>0.182 ± 0.087*</td>
</tr>
<tr>
<td>(L(4,3))</td>
<td>0.189 ± 0.103</td>
<td>0.489 ± 0.217*</td>
</tr>
<tr>
<td>(L(3,4))</td>
<td>0.0849 ± 0.0494</td>
<td>0.224 ± 0.066*</td>
</tr>
<tr>
<td>(L(31,3))</td>
<td>0.00789 ± 0.00422</td>
<td>0.0284 ± 0.0114*</td>
</tr>
<tr>
<td>(L(32,3))</td>
<td>0.0499 ± 0.0185</td>
<td>0.145 ± 0.0480*</td>
</tr>
</tbody>
</table>

* Significantly different from controls \((p < 0.05)\).

### TABLE 4

Kinetic Parameters for Vitamin A Kinetics in Control and TCDD-Treated Rats in Experiment 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r(1)) (h)</td>
<td>1.34 ± 0.34</td>
<td>1.02 ± 0.17</td>
</tr>
<tr>
<td>(r(2)) (h)</td>
<td>1.75 ± 0.52</td>
<td>1.38 ± 0.45</td>
</tr>
<tr>
<td>(r(3)) (days)</td>
<td>3.51 ± 1.85</td>
<td>1.39 ± 0.61*</td>
</tr>
<tr>
<td>(r(4)) (days)</td>
<td>15.4 ± 7.9</td>
<td>4.94 ± 1.82*</td>
</tr>
<tr>
<td>(T(1)) (day)</td>
<td>0.613 ± 0.194</td>
<td>0.514 ± 0.073</td>
</tr>
<tr>
<td>(T(2)) (day)</td>
<td>0.594 ± 0.193</td>
<td>0.556 ± 0.090</td>
</tr>
<tr>
<td>(T(3)) (days)</td>
<td>18.2 ± 5.39</td>
<td>6.38 ± 2.36*</td>
</tr>
<tr>
<td>(T(4)) (days)</td>
<td>49.0 ± 28.6</td>
<td>13.1 ± 3.8*</td>
</tr>
<tr>
<td>(T(SYS)) (days)</td>
<td>68.4 ± 32.9</td>
<td>20.5 ± 4.8*</td>
</tr>
<tr>
<td>(u(I))</td>
<td>8.38 ± 1.70</td>
<td>11.2 ± 2.3*</td>
</tr>
<tr>
<td>(u(2)) (days)</td>
<td>5.41 ± 0.80</td>
<td>1.86 ± 0.97*</td>
</tr>
<tr>
<td>FCRp (\text{days}^{-1})</td>
<td>1.74 ± 0.43</td>
<td>1.98 ± 0.32</td>
</tr>
<tr>
<td>DR (\text{nmol/day})</td>
<td>47.9 ± 8.4</td>
<td>60.8 ± 10.3*</td>
</tr>
</tbody>
</table>

* Significantly different from controls \((p < 0.05)\).
3, top), as in experiment 1, plasma response profiles for TCDD-treated rats showed an earlier bend (beginning at ~1 day) and a much steeper terminal slope, compared to controls.

Essentially all of the administered radioactivity (>94%) was recovered in plasma + liver + small intestine + carcass at 25 min in both groups. Recovery of the radioactive dose in small intestines was low in the short-term rats from both groups, averaging 0.4–1% at 25 min and 1.8–2.6% at 8 h. This result indicates that essentially none of the chylomicron dose had been cleared via the liver reticuloendothelial system and excreted into bile and small intestine, as it might have been if the dose had contained a nonphysiological component.

Hepatic recovery of the radioactive dose is shown in Table 5. At all times, the majority of the radioactivity in liver was in retinyl esters. For control rats, hepatic recovery at 8 h was lower than that at 25 min. At 8 h and 2 days, hepatic recoveries were significantly lower in livers of TCDD-treated versus control rats. By 42 days, differences between the groups were even more marked than in experiment 1, with only 0.6% of the dose recovered in livers of TCDD-treated rats versus 46% in controls.

Recovery of the injected radioactive dose in carcass (Table 5) was similar for TCDD-treated and control rats at 25 min, whereas recovery tended to be higher at 8 h (18%) and it was

![Graphs showing plasma tracer response profiles for control and TCDD-treated rats](image-url)

**FIG. 3.** Plasma tracer response profiles for a representative control (□) and TCDD-treated rat (Δ) for 42 days (top) after administration of [3H]vitamin A-labeled lymph chylomicrons (experiment 2) and irreversible loss on day 42 (control, O; TCDD V). Also shown are the first day's plasma response on an expanded scale (middle) and fraction of dose in plasma retinol (control, O; TCDD, V) and retinyl esters (control, □; TCDD, Δ) during the first 2 h (bottom). Lines are the responses predicted by the compartmental model shown in Fig. 4.

Differences between controls and TCDD-treated rats were evident even at very early times after dosing. Specifically, there was an earlier bend in the plasma tracer response curve between 10 and 30 min in the TCDD-treated rat (Fig. 3, middle, dotted line), a later peak in plasma retinol radioactivity (215–260 min), and wider appearance of the “hump.” At later times (Fig.

**TABLE 5**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 min Total</td>
<td>0.762 ± 0.018</td>
<td>0.766 ± 0.038</td>
</tr>
<tr>
<td>RE</td>
<td>0.580 ± 0.014</td>
<td>0.616 ± 0.035</td>
</tr>
<tr>
<td>8 h Total</td>
<td>0.438 ± 0.067</td>
<td>0.258 ± 0.018*</td>
</tr>
<tr>
<td>RE</td>
<td>0.382 ± 0.057</td>
<td>0.194 ± 0.025*</td>
</tr>
<tr>
<td>2 days Total</td>
<td>0.633 ± 0.034</td>
<td>0.235 ± 0.034*</td>
</tr>
<tr>
<td>RE</td>
<td>0.576 ± 0.032</td>
<td>0.208 ± 0.002*</td>
</tr>
<tr>
<td>42 days Total</td>
<td>0.462 ± 0.073</td>
<td>0.0061 ± 0.0022*</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 min</td>
<td>0.134 ± 0.034</td>
<td>0.092 ± 0.003</td>
</tr>
<tr>
<td>8 h</td>
<td>0.317 ± 0.029</td>
<td>0.375 ± 0.069</td>
</tr>
<tr>
<td>2 days</td>
<td>0.152 ± 0.018</td>
<td>0.252 ± 0.030*</td>
</tr>
<tr>
<td>42 days</td>
<td>0.079 ± 0.009</td>
<td>0.047 ± 0.013*</td>
</tr>
<tr>
<td>Cumulative urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>0.026 ± 0.004</td>
<td>0.075 ± 0.009*</td>
</tr>
<tr>
<td>Cumulative feces</td>
<td>0.050 ± 0.007</td>
<td>0.139 ± 0.026*</td>
</tr>
</tbody>
</table>

Note. Data are means ± SD for fraction of administered dose of [3H]vitamin A-labeled lymph chylomicrons recovered in total liver, liver retinyl esters (RE), carcass, urine, and feces. Urine and feces were collected as one 4-day pool from the time of administration of label. N = 3 for control and TCDD-treated rats killed at 25 min, 8 h, and 2 days after administration of label, n = 8 for controls killed at 42 days, and n = 7 for TCDD-treated rats at 42 days. *Significantly different from controls (p < 0.05).
significantly higher (66%) at 2 days in TCDD-treated rats than in control rats. By the end of the experiment, recovery of the labeled dose in carcass had decreased to levels lower than those observed in experiment 1 and was significantly lower in TCDD-treated rats than in controls.

As in experiment 1, TCDD treatment was associated with significant increases in urinary and fecal excretion of labeled vitamin A, and more of the dose radioactivity was excreted in feces than in urine (Table 5). At 4 days after administration of the labeled dose, cumulative recovery of radioactivity was almost three times higher in urine and feces of TCDD-treated versus control rats. Although >94% of the administered radioactivity was recovered at 25 min, at later times, the recovery was lower due to metabolism and excretion of the label. For example, recovery in plasma + liver + carcass + small intestine at 2 days averaged 80.7 ± 1.6% (n = 3) in control rats and 51.7 ± 3.6% (n = 2) in TCDD-treated animals. Assuming that excretion of radioactivity in urine and feces at 2 days was less than that observed at 4 days, some of the dose (~15% in controls and 20% in TCDD-treated rats) had already been converted to nonextractable polar metabolites. The nonrecovery of such polar metabolites would not alter our kinetic analyses or results, since, once retinol is metabolized to retinoic acid or other polar metabolites, it cannot recycle to plasma retinol and is thus irreversibly utilized from the kinetic perspective. Still, additional useful information could be obtained in future studies by using a biological materials oxidizer to combust samples of carcass and feces to $^3$H$_2$O prior to liquid scintillation spectrometry.

**Experiment 2: Model Development and Kinetic Parameters**

We began model development for experiment 2 data using the four-compartment model developed in experiment 1, after addition of compartments needed to account for the introduction of the label on lymph chylomicrons (Fig. 4). In view of the distribution of label in lymph, 90.5% of the dietary input into the system [U(I)] is shown into the plasma retinyl ester input compartment (compartment 5) and 9.5% into a chylomicron retinol compartment (compartment 11). In order to explain the complex geometry of the initial portion of the curves and based on current understanding of the metabolism of vitamin A in chylomicrons, a fraction of labeled chylomicron retinyl esters was transferred from compartment 5 to a rapidly turning-over extravascular compartment (compartment 7) that was needed to fit early data for $^3$H-retinyl esters in plasma. To fit the later rise in labeled plasma retinol, a delay element (component 6), presumably in the liver, was also required. The model requires that, after processing through compartment 7 and component 6 (which together probably represent liver uptake of chylomicron-derived retinyl esters, hydrolysis of the esters, and transfer of retinol to apoRBP), labeled retinol is released into plasma (compartment 1) bound to RBP. Labeled retinol in the administered dose (compartment 11) was processed through a single extravascular compartment (compartment 9) before transfer into plasma compartment 1. As in the model developed for experiment 1, once the label was secreted into plasma, it was able to exchange with vitamin A in both a rapidly turning-over extravascular compartment (compartment 2) and a more slowly turning-over extravascular compartment (compartment 3) before leaving the system irreversibly through compartment 3. Since less extensive information on urinary and fecal output of radioactivity was collected in the second experiment, the model shows only one site of output [L(0,3)]. Also, in contrast to the model developed for experiment 1, a second slowly turning-over extravascular compartment was not statistically justified.

As in experiment 1, tracer data for each rat were fit to this model by adjusting the values for the fractional transfer coefficients [L(I,J)s]. As is evident in Fig. 3 by comparing observed
treated versus control rats. TCDD caused significant reductions
determined in experiment 1 and were not different for TCDD-
treatment 7 \( f(7) \) was 49% lower after TCDD exposure. Mean
was irreversibly lost from the system \( L(0,3) \) was 3.6 times
doubled in TCDD-treated rats. Finally, the fraction of label that
parted from compartments 2 and 3 were both more than
metabolism of retinol/RBP, the processing of the
H\]retinol bound to RBP, the processing of the
3
H\]vitamin A-labeled lymph chylomicrons
Treated Rats in Experiment 2
Proposed Model of Vitamin A Metabolism in Control and TCDD-
Control and TCDD-Treated Rats in Experiment 2
TCDD-Treated Rats in Experiment 2

**TABLE 6**
Model-Predicted Fractional Transfer Coefficients \( L(I,J)\)s for Proposed Model of Vitamin A Metabolism in Control and TCDD-Treated Rats in Experiment 2

<table>
<thead>
<tr>
<th>( L(I,J) )</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L(7,5) ) (days(^{-1} ))</td>
<td>185 ± 48</td>
<td>240 ± 118</td>
</tr>
<tr>
<td>( L(5,7) ) (days(^{-1} ))</td>
<td>1.82 ± 0.72</td>
<td>13.8 ± 12.2*</td>
</tr>
<tr>
<td>( L(6,7) ) (days(^{-1} ))</td>
<td>6.10 ± 0.84</td>
<td>10.8 ± 5.1*</td>
</tr>
<tr>
<td>( DT(6) ) (min)</td>
<td>37.3 ± 3.4</td>
<td>56.4 ± 11.3*</td>
</tr>
<tr>
<td>( L(9,11) ) (days(^{-1} ))</td>
<td>222 ± 67</td>
<td>258 ± 93</td>
</tr>
<tr>
<td>( L(1,9) ) (days(^{-1} ))</td>
<td>6.10 ± 1.79</td>
<td>6.96 ± 3.73</td>
</tr>
<tr>
<td>( L(2,1) ) (days(^{-1} ))</td>
<td>12.2 ± 3.8</td>
<td>16.9 ± 8.1</td>
</tr>
<tr>
<td>( L(1,2) ) (days(^{-1} ))</td>
<td>2.48 ± 1.44</td>
<td>5.86 ± 2.98*</td>
</tr>
<tr>
<td>( L(3,1) ) (days(^{-1} ))</td>
<td>15.4 ± 3.9</td>
<td>13.6 ± 3.4</td>
</tr>
<tr>
<td>( L(1,3) ) (days(^{-1} ))</td>
<td>0.0931 ± 0.0169</td>
<td>0.210 ± 0.048*</td>
</tr>
<tr>
<td>( L(0,3) ) (days(^{-1} ))</td>
<td>0.0200 ± 0.0042</td>
<td>0.0743 ± 0.0101*</td>
</tr>
</tbody>
</table>

**Note.** Values are population mean fractional transfer coefficients \( L(I,J)\)s, or the fraction of compartment J's tracer transferred to compartment I each day ± estimated population standard deviation for control \( n = 8 \) and TCDD-treated rats \( n = 7 \) after administration of \(^{3}H\) vitamin A labeled lymph chylomicrons in experiment 2. \( L(I,J)\)s describe the model presented in Fig. 4.

* Significantly different from controls \((p < 0.05)\).

---

TABLE 7
Kinetic Parameters for Vitamin A Kinetics in Control and TCDD-Treated Rats in Experiment 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha(5) ) (h)</td>
<td>0.139 ± 0.042</td>
<td>0.130 ± 0.072</td>
</tr>
<tr>
<td>( \alpha(7) ) (h)</td>
<td>6.96 ± 3.73</td>
<td></td>
</tr>
<tr>
<td>( \alpha(9) ) (h)</td>
<td>4.27 ± 1.28</td>
<td>4.31 ± 2.00</td>
</tr>
<tr>
<td>( \alpha(11) ) (h)</td>
<td>0.120 ± 0.048</td>
<td>0.105 ± 0.037</td>
</tr>
<tr>
<td>( \alpha(1) ) (h)</td>
<td>0.894 ± 0.269</td>
<td>0.837 ± 0.232</td>
</tr>
<tr>
<td>( \alpha(2) ) (h)</td>
<td>12.2 ± 5.8</td>
<td>5.29 ± 3.00*</td>
</tr>
<tr>
<td>( \alpha(3) ) (days)</td>
<td>9.05 ± 1.58</td>
<td>3.62 ± 0.69*</td>
</tr>
<tr>
<td>( \alpha(T) ) (day)</td>
<td>0.381 ± 0.029</td>
<td>0.295 ± 0.061*</td>
</tr>
<tr>
<td>( \alpha(T) ) (days)</td>
<td>2.28 ± 0.73</td>
<td>0.939 ± 0.484*</td>
</tr>
<tr>
<td>( \alpha(T) ) (days)</td>
<td>52.3 ± 11.7</td>
<td>13.8 ± 2.2*</td>
</tr>
<tr>
<td>( \alpha(T) ) (days)</td>
<td>55.2 ± 11.9</td>
<td>15.2 ± 2.2*</td>
</tr>
<tr>
<td>( \alpha(1) ) (h)</td>
<td>8.55 ± 2.62</td>
<td>8.10 ± 3.15</td>
</tr>
<tr>
<td>( \alpha(11) ) (h)</td>
<td>5.61 ± 1.34</td>
<td>2.10 ± 0.85*</td>
</tr>
<tr>
<td>( \alpha(T) ) (days)</td>
<td>2.60 ± 0.19</td>
<td>3.50 ± 0.63*</td>
</tr>
<tr>
<td>( \alpha(T) ) (days)</td>
<td>55.3 ± 6.6</td>
<td>84.1 ± 17.1*</td>
</tr>
</tbody>
</table>

**Note.** Values are calculated kinetic parameters \((\text{means} ± \text{SD})\) for control \((n = 8)\) and TCDD-treated rats \((n = 7)\). The model is presented in Fig. 4. Parameters are transit times \( T(1) \), or the mean of the distribution of times that retinol molecules entering compartment I remain in compartment I during a single transit before leaving reversibly or irreversibly, where \( T(1) = 1/\alpha(11) \); residence times \( T(1) \), or the mean of the distribution of times that retinol molecules spend in compartment I after entering the system via compartment J before irreversibly leaving compartment I, are equal to the area under the tracer response curve for compartment I integrated from 0 to infinity \( \Upsilon(U) \); \( T(U) \), system residence time, or the length of time the average vitamin A molecule spends in the system before irreversible loss, where \( T(U) = \Upsilon(U) \); recycling number \( \Upsilon(U) \), or the average number of times a molecule of retinol cycles through compartment I before leaving compartment I irreversibly, where \( \Upsilon(U) = \Upsilon(U) \); disposal rate \( \Upsilon(U) \), or the fraction of the plasma vitamin A pool that is irreversibly utilized each day, where \( \Upsilon(U) = U(U)M(U) \), where \( U(U) \) is the rate of input of trace into compartment I; and \( DR \), disposal rate, or the daily utilization rate for vitamin A where \( DR(U) = FCR(U) \) ± M(U).

* Significantly different from controls \((p < 0.05)\).

---

Additional kinetic parameters for experiment 2 are presented in Table 7. The mean transit time for vitamin A in compartment 1 \( T(1) \) was 49% lower after TCDD exposure. Mean transit times in compartment 1 \( T(1) \) were similar to those determined in experiment 1 and were not different for TCDD-treated versus control rats. TCDD caused significant reductions (57 and 60%) in the transit times in compartments 2 and 3, respectively. The mean residence times \( T(1) \) in compartments 1–3 were all significantly reduced with TCDD treatment. Of these perturbations, the most dramatic effect was seen in compartment 3, where TCDD caused a 74% reduction in mean residence time. The reduced residence time in compartment 3 was the most significant contributor to the 73% lower system residence time \( T(SYS) \) in the TCDD-treated rats; this reduction is comparable to that calculated for experiment 1. Vitamin A recycling time \( T(1) \) was significantly lower (63%) in TCDD-treated rats, whereas the plasma fractional catabolic rate \( FCR(U) \) and the disposal rate \( DR \) were significantly higher (35 and 52%, respectively) in TCDD-treated versus control rats.
DISCUSSION

As summarized in the introduction and in a review by Zile (1992), TCDD exposure has a variety of adverse effects on vitamin A metabolism in rats. Here we investigated the mechanisms involved in the effects of TCDD exposure on vitamin A metabolism by using model-based compartmental analysis to determine which whole-body vitamin A kinetic processes are perturbed by TCDD treatment in the rat.

Based on work by Håkansson and Ahlborg (1985) and Håkansson and Hanberg (1989), which showed that TCDD administration affects the storage and excretion of newly absorbed vitamin A, we speculated that TCDD might disrupt vitamin A homeostasis by altering the uptake and processing of newly absorbed retinyl esters or by affecting the normal, extensive recycling of retinol among plasma, liver, and extrahepatic tissues that has been documented by previous compartmental models (Green et al., 1985, 1987). Our results from experiment 2 indicate that there are subtle effects of TCDD treatment on chylomicron clearance, as well as on the rate of processing of chylomicron-derived vitamin A in the liver. However, by 1 day, the model (Fig. 4) predicts that in both groups, the liver had processed essentially all of the labeled incoming chylomicron vitamin A. Thus, it seems unlikely that the dramatic effects of TCDD on vitamin A metabolism can be attributed to alterations in the metabolism of chylomicrons and/or the initial hepatic handling of chylomicron-derived vitamin A. Additionally, results from both experiments indicate that there is no effect of TCDD on the fractional uptake of RBP-retinol by tissues [L(2,1) and L(3,1), Tables 3 and 6].

Although tissue uptake of vitamin A from plasma was unaffected by TCDD treatment, the models predict that TCDD increases the fractional recycling of vitamin A from faster and more slowly turning-over pools into plasma (Table 3). Liver vitamin A balance data (Table 1) are compatible with the idea that, as liver vitamin A levels decrease (by ~90%) during the turnover study in TCDD-treated rats, a larger fraction of the remaining pool is mobilized to maintain a constant rate of turnover study in TCDD-treated rats, a larger fraction of the remaining pool is mobilized to maintain a constant rate of turnover.

Regarding effects of TCDD on degradation of vitamin A, our data (Tables 2 and 5) and earlier studies (Håkansson and Ahlborg, 1985; Håkansson et al., 1988) show that TCDD treatment results in increased excretion of vitamin A metabolites in urine and feces. Our models predict significant elevations in the irreversible utilization of vitamin A in TCDD-treated versus control rats (Tables 4 and 7). The idea (Moore, 1957; Wolf, 1984; Håkansson et al., 1988; Green and Green, 1996) that vitamin A utilization is composed of both a functional (related to the biological activity of vitamin A) and a nonfunctional component (a mechanism for ridding the body of vitamin A through degradation) may be relevant to these observed effects of TCDD exposure. First, it is difficult to imagine a functional process that could account for the magnitude of the depletion in liver vitamin A observed in TCDD-treated rats. Further, work by others (Bank et al., 1989; Fiorella et al., 1995) supports the idea of increases in nonfunctional catabolism, possibly related to TCDD induction of cytochrome P-450 enzymes [see reviews by Zile (1992) and Pohjanvirta and Tuomisto (1994)]. Such an increase in catabolism might result not only in a decrease in the availability of intracellular retinoic acid for vitamin A action, but also a need for further mobilization from retinyl ester stores.

Due to the significant overlap in the consequences of TCDD toxicity and vitamin A deficiency, it has been suggested (Zile, 1992) that some of the toxic symptoms of TCDD exposure may be due to a functional vitamin A deficiency in target tissues. However, the plasma retinol turnover rate for TCDD-treated rats in experiment 1 was ~10 times higher than that predicted for the vitamin A-deficient rats studied by Lewis et al. (1990). In addition, rats with low vitamin A status had very low vitamin A disposal rates (5.8 nmol/day versus >60 nmol/day in TCDD-treated rats); and residence times for retinol in plasma and liver were much lower than those in TCDD-treated rats. Our results suggest that, at the whole-body level, vitamin A dynamics are dramatically different in TCDD-treated versus vitamin A-deficient rats.

We used model-predicted fractional transfer coefficients [L(I,J)s] to estimate compartment vitamin A masses [M(I)] and...
rates (nmol/day) of vitamin A transfer between compartments and into and out of the system. This was done by adjusting the input rates and the transfer rates related to storage in order to reflect the observed vitamin A balances (net storage for control rats or net mobilization for TCDD-treated rats) (Fig. 5). That is, we assumed a vitamin A steady state in all compartments except the most slowly turning-over storage compartments (compartment 4 in experiment 1 and compartment 3 in experiment 2). This analysis indicates that, although control and TCDD-treated rats consumed the same diet, TCDD-treated animals absorbed ~40 nmol/day less vitamin A than controls. TCDD exposure is frequently associated with a decrease in food intake (Pohjanvirta and Tuomisto, 1994) and in fact, our data on differences in body weight gain between TCDD-treated and control rats (Table 1) may indicate that there was a decrease in food intake. Our calculations suggest that a small decrease in food intake, coupled with a decrease in vitamin A absorption efficiency, results in a vitamin A input into the system which is 40–50% lower in TCDD-treated than control rats. In other work in this lab, vitamin A absorption tended to be lower in lymph duct-cannulated rats given a single oral dose of TCDD (Hanberg et al., submitted for publication). In spite of an estimated decrease in vitamin A absorption, we would have predicted, based on estimates (Green et al., 1985, 1987) of vitamin A utilization in normal adult rats, that TCDD-treated rats in experiment 1 should have been in vitamin A balance and those in experiment 2 in positive balance. Since, in contrast, the data indicate that TCDD-treated rats in both experiments were in negative vitamin A balance, we conclude that TCDD affects vitamin A utilization (24–52% higher in TCDD-treated rats) independently of its effects on food intake and vitamin A absorption efficiency.

The rate of movement of vitamin A into slow turning-over storage compartments (compartment 4 in experiment 1 and compartment 3 in experiment 2) was not dramatically affected by TCDD treatment, especially in view of the predicted differences in vitamin A input. However, mobilization from those pools was high, resulting in a negative vitamin A balance in the liver of TCDD-treated rats. As argued above, TCDD may divert vitamin A into catabolic processes. Perhaps this diversion decreases the availability of retinol in a pool that is involved in the homeostatic regulation of retinol ester hydrolysis. Since dietary supplementation with retinoic acid (Keilson et al., 1979) (and perhaps consequently cellular concentrations of retinoic acid) and the ratio of apo/holo cellular retinol-binding protein (CRBP) (Herr and Ong, 1992) have been shown to influence the mobilization of vitamin A from storage pools and the formation of retinyl esters, it would be interesting to study effects of TCDD on cellular retinoic acid, apo/holo CRBP ratios, and CRBP levels.

In conclusion, this paper presents the first application of model-based compartmental analysis to exploring the disruption of vitamin A homeostasis by TCDD. Our results indicate that the primary sites of the dysregulation by TCDD are increased catabolism and mobilization of vitamin A from slowly turning-over tissue stores of vitamin A (presumably mainly the liver). Based on our data, we are not able to say whether the increased utilization results in increased mobilization or whether the increased mobilization leads to increased utilization. Since past work and the current results suggest that the liver is a main target for TCDD's effects on vitamin A metabolism, it would be informative to characterize in detail the effects of TCDD on vitamin A dynamics in the liver, using an approach similar to that described by Green et al. (1993). It would also be interesting to use kinetic approaches to study TCDD-related changes in renal vitamin A dynamics, in order to determine whether the reciprocal effects of TCDD on liver and kidney might be due to an alteration in signaling among kidneys, plasma, and liver.

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REFERENCES

EFFECTS OF TCDD ON VITAMIN A KINETICS


