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 μg/kg) followed by weekly maintenance doses (0.7 μg/kg) or oil only. [3H]Retinol in its plasma transport complex (experiment 1) or lymph containing chylomicrons labeled mainly with [3H]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$^+$ 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 xenobiotic 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 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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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 rats and those exposed to weekly doses of TCDD after administration of a radioactive (tracer) dose of vitamin A. 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 photooxidation 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-H) 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 short-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)/transthyretin (TTR)) 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/TTR (Green et al., 1985) was stored under an atmosphere of nitrogen at 4°C and used for kinetic studies over the next 2 days. Weighed 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 livers were 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, blotted, 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 intraperitoneally with an accurately weighed amount (−0.4 g containing −180 kBq) of [3H]retinol-labeled plasma. The

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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 minivalves 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 (20–24 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 weighed and aliquots (3X ~1 g) were weighed
into minivalves and frozen for later analysis of tritium (see below). Feces were
transferred from their collection cylinder into sample bags, weighed, frozen,
yoprylilized, 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 thymus glands were
excised and weighed (so that effects of TCDD on thymus 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
TBq of [11,12(N)3H]retinol (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 analyzed for radioactivity in
total 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
(0.125 g containing ~120 kBq). Rats were designated as either short-term or
long-term recipients. For rats in the short-term group (TCDD, n = 7; control,
= 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 analysis of tritium and, at selected times, for retinol concentration (see
below). In addition, aliquots of plasma were collected from the first 2 h after
dosing were frozen for analysis of tritium in retinol versus retinyl esters (see below).

**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, Hiltne, Stockholm, Sweden). Samples were counted twice to a
final 2σ 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 quanti-
tify 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/LabKemi, Stockholm, Sweden)
deactivated with 5% water (Ross, 1982). Retinyl esters were eluted with 3% dietyholether in hexane and retinol with 50% dietyholether. Fractions were ana-
talyzed for tritium (LS 180; Beckman Instruments, Stockholm, Sweden)
using Ecoscint O (National Diagnostics) as scintillation solution.

Triplicate aliquots of freeze-dried liver (0.15 g) were superfused in ethanolic
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 ana-
lyzed 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 Hara and Radin (1978) as de-
scribed 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, Uppsala, Sweden) after addition of Ecoscint A to triplicate
aliquots of urine. Conversion of net cpm to dpm for each sample was done
using an external standard channels ratio method.

Fecal radioactivity was determined using a modification of the method of
Håkansson and Ahlborg (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. Sol-
vent 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 (f dose) 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 reversible 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(I)] is the length of time an average molecule of tracer spends in compartment I during a single transit. It was calculated as 1/2L(IJ). T(IJ), 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 1; it is equal to the area under the tracer response curve integrated from 0 to infinity [AUC(IJ)]. Plasma fractional catabolic rate [FCR,] or the fraction of the plasma retinol pool that is irreversibly utilized each day, was calculated as 1/T(I). The number of times that a retinol molecule recycles through compartment I before irreversible loss is the recycling number [n(I)]. It was calculated as [T(I)/T(I)] – 1. T(SYS), or system mean residence time, expressed by the equation T(SYS) = ΣT(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 [n(I)]; it was calculated as T(SYS) – T(IJ)/x). 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.
in response profiles, we began model development using the three-vitamin A levels, and the geometry of the 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 to plasma from rapidly 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 extracellular 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
significantly higher (43%) in TCDD-treated rats; die transfer to the rapidly turning-over extravascular compartment, was sig-
ificant. L(2,1), the fractional transfer of retinol from plasma to ri-
able materials and Mediods), group mean fractional transfer coefficients was less than 0.5 and in most cases, they were less than 0.15.

tional standard deviations for each $L(I,J)$ in each rat's model were well identified, based on the criterion that the estimated frac-
tional 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 Mate-
rials and Methods), group mean fractional transfer coefficients $[L(I,J)]s$ and their statistical uncertainties were calculated (Ta-
ble 3). $L(2,1)$, the fractional transfer of retinol from plasma to the rapidly turning-over extravascular compartment, was signifi-
cantly higher (43%) in TCDD-treated rats; the transfer to

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 $[1 - (\text{fraction of dose in plasma + liver + carcass})]$ 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.

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

\[ \frac{dQ_{i}}{dt} = L(1,2)Q(2) + L(1,3)Q(3) - \{L(3,1)Q(1) + L(2,1)Q(1)\}, \]

where $Q_{i}$ equals the amount of labeled vitamin A in compartment $i$ and $L(I,J)s$ are fractional transfer coefficients.

FIG. 2. Working compartmental model developed to fit tracer data for plasma, carcass, irreversible loss $[1 - (\text{fraction of dose in plasma + liver + carcass})]$, 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

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>
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<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>0.099 ± 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 radio-
activity 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 ($p < 0.05$).
In order to examine effects of TCDD exposure on the metabolism of dietary vitamin A, rats in experiment 2 were injected with [3H]retinyl ester-labeled lymph chylomicrons. Plasma tracer response curves for a representative control and TCDD-treated rat 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 [3H]retinyl esters primarily by liver. Then, plasma radioactivity rose from ~30 min to a peak at 140 min, corresponding to liver secretion of [3H]retinol/RBP/TTR into plasma. Subtle differ-

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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

**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 RE</td>
<td>0.438 ± 0.067</td>
<td>0.258 ± 0.018*</td>
</tr>
<tr>
<td>2 days Total RE</td>
<td>0.382 ± 0.057</td>
<td>0.194 ± 0.024**</td>
</tr>
<tr>
<td>42 days Total RE</td>
<td>0.576 ± 0.032</td>
<td>0.208 ± 0.002*</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>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).
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) and 9.5% into compartment 11 as retinol) and U(5) represents dietary input into the system. As an example, the differential equation used to describe the change in labeled vitamin A present in compartment 1 is \( \frac{dQ_1}{dt} = L(1,6)Q(6) + L(1,9)Q(9) - \left[ L(3,1)Q(1) \right] \), where \( Q_i \) equals the amount of labeled vitamin A in compartment 1 and \( L(I,J) \) are fractional transfer coefficients.

As in experiment 1, tracer data for each rat were fit to this model by adjusting the values for the fractional transfer coefficients \( L(I,J) \). As is evident in Fig. 3 by comparing observed significantly higher (66%) at 2 days in TCDD-treated 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.
data (symbols) to the model predictions (lines), the model provided a good fit to plasma tracer data. All parameters were well identified (estimated parameter FSDs < 0.5), with the highest variability seen in L(5,7). Group mean fractional transfer coefficients and their statistical uncertainties are shown in Table 6. Several parameters associated with metabolism of the chylomicron dose [L(5,7), L(6,7), and DT(6)] were significantly higher in TCDD-treated than in control rats. The increase in L(5,7) suggests that a higher fraction of the label that reaches the liver on absorptive lipoproteins is recycled to plasma before hepatic uptake in TCDD-treated rats (56% versus 23% in controls). The increase in L(6,7) in the TCDD group indicates that, after internalization, label was more rapidly transferred to the delay component. Before secretion into plasma as [3H]retinol bound to RBP, the processing of the labeled diet-derived vitamin A in liver [DT(6)] took 51% longer in TCDD-treated rats. For parameters related to metabolism of retinol/RBP, fractional transfers of retinol from plasma compartment 1 to extravascular compartments 2 and 3 [L(2,1) and L(3,1), Table 6] were unaffected by TCDD treatment but the L(I,J)s reflecting recycling to the plasma compartment from compartments 2 and 3 were both more than doubled in TCDD-treated rats. Finally, the fraction of label that was irreversibly lost from the system [L(0,3)] was 3.6 times higher in animals given TCDD than that in controls.

Additional kinetic parameters for experiment 2 are presented in Table 7. The mean transit time for vitamin A in compartment 7 [τ(7)] was 49% lower after TCDD exposure. Mean transit times in compartment 1 [τ(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(I)] 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 [τ(r)] was significantly lower (63%) in TCDD-treated rats, whereas the plasma fractional catabolic rate (FCR_p) and the disposal rate (DR) were significantly higher (35 and 52%, respectively) in TCDD-treated versus control rats.

<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>12.6 ± 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 [3H]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 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>Parameter</th>
<th>Control</th>
<th>TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>τ(5) (h)</td>
<td>0.139 ± 0.042</td>
<td>0.130 ± 0.072</td>
</tr>
<tr>
<td>τ(7) (h)</td>
<td>3.07 ± 0.46</td>
<td>1.50 ± 1.05</td>
</tr>
<tr>
<td>τ(9) (h)</td>
<td>4.27 ± 1.28</td>
<td>4.31 ± 2.00</td>
</tr>
<tr>
<td>τ(11) (h)</td>
<td>0.120 ± 0.048</td>
<td>0.105 ± 0.037</td>
</tr>
<tr>
<td>τ(1) (h)</td>
<td>0.894 ± 0.269</td>
<td>0.837 ± 0.232</td>
</tr>
<tr>
<td>τ(2) (h)</td>
<td>12.2 ± 5.8</td>
<td>5.29 ± 3.0*</td>
</tr>
<tr>
<td>τ(3) (days)</td>
<td>9.05 ± 1.58</td>
<td>3.62 ± 0.69*</td>
</tr>
<tr>
<td>τ(T) (day)</td>
<td>0.381 ± 0.029</td>
<td>0.295 ± 0.061*</td>
</tr>
<tr>
<td>τ(T2) (days)</td>
<td>2.28 ± 0.73</td>
<td>0.939 ± 0.484*</td>
</tr>
<tr>
<td>τ(T3) (days)</td>
<td>52.3 ± 11.7</td>
<td>13.8 ± 2.2*</td>
</tr>
<tr>
<td>τ(TSYS) (days)</td>
<td>55.2 ± 11.9</td>
<td>15.2 ± 2.2*</td>
</tr>
<tr>
<td>τ(x) (l)</td>
<td>8.55 ± 2.62</td>
<td>8.10 ± 3.15</td>
</tr>
<tr>
<td>τr (l) (days)</td>
<td>5.61 ± 3.14</td>
<td>2.10 ± 0.85*</td>
</tr>
<tr>
<td>FCR_p (days⁻¹)</td>
<td>2.60 ± 0.19</td>
<td>3.50 ± 0.63*</td>
</tr>
<tr>
<td>DR (nmol/day)</td>
<td>55.3 ± 6.6</td>
<td>84.1 ± 17.1*</td>
</tr>
</tbody>
</table>

**Note.** Values are calculated kinetic parameters (means ± SD) for control (n = 8) and TCDD-treated rats (n = 7). The model is presented in Fig. 4. Parameters are transit times [τ(I)], 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 [τ(I)] = 1/ΣL(I,J); residence times [T(I,J)], 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 [AUC(I)]; T(SYS), system residence time, or the length of time the average vitamin A molecule spends in the system before irreversible loss, where T(SYS) = ΣT(I,J); recycling number [x(I)], or the average number of times a molecule of retinol recycles through compartment I before leaving compartment I irreversibly, where [x(I)] = τ(SYS)/τ(I); recycling rate [τr(l)], or the time it takes for the average retinol molecule leaving compartment I to return to compartment I, where [τr(l)] = T(SYS) - τ(I,J); FCR_p, the plasma fractional catabolic rate, or the fraction of the plasma retinol pool that is irreversibly utilized each day, where FCR_p = U(I)/M(I), where U(I) is the rate of input of trace into compartment I; and DR, disposal rate, or the daily utilization rate for vitamin A where DR(SYS) = FCR_p * M(I).

* Significantly different from controls (p < 0.05).
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 tissue [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 retinol secretion into plasma, resulting in stable (although slightly increased) plasma retinol concentrations. Although interesting, this increased mobilization of vitamin A into plasma does not explain how TCDD might be interfering with vitamin A storage in liver (Håkansson and Ahlborg, 1985; Håkansson et al., 1988) and liver stellate cells (Håkansson and Hanberg, 1989; Nilsson et al., 1996).

Based on a study by Nilsson et al. (1996), which showed that TCDD decreases both lecithin:retinol acyltransferase and acyl CoA:retinyl acyltransferase activity in hepatic stellate cells, we expected to see a lower transfer of label into vitamin A storage pools in experiment 1. However, our kinetic results indicate that vitamin A is moved into storage pools more rapidly with TCDD treatment and that it is also removed more quickly. That is, there may be a futile cycling of vitamin A between compartments 3 and 4, involving increased formation or storage of retinyl esters, and an increased net mobilization of vitamin A (most likely through increased hydrolysis in conjunction with increased degradation). Although Brouwer et al. (1988) found no influence of TCDD on activity of the bile salt-dependent retinyl ester hydrolase (REH) in rat liver homogenates, there is the possibility that the membrane-bound, neutral REH activity, which is present in rat liver stellate cells as well as hepatocytes (Matsuura et al., 1997), or the acid REH could be involved. In this regard, Nilsson et al. (unpublished data) have found that the activity of membrane-bound, neutral REH activity in whole liver, as well as isolated stellate cells and hepatocytes, is not affected by TCDD treatment. It is worth noting that increased hydrolysis need not require an increase in the amount of REH protein, but might result from an increase in relative activity due to the removal of product.

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 retinyl 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/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


