**Interaction between Dietary Vitamin A and Single Oral Doses
of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)
on the TCDD-Induced Toxicity and on the
Vitamin A Status in the Rat¹**

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Summary The effect of dietary vitamin A on the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the effect of TCDD on the tissue vitamin A levels in 8-week-old male Sprague-Dawley rats of variable vitamin A status, were investigated. Rats were fed purified casein based diets containing 2,000, 5,000, 8,000, or 21,000 IU of vitamin A/kg from weaning and throughout the experiment. The study was terminated 44 days after the administration of a single oral dose of 0, 15, 30, 60, or 120 μ g TCDD/kg body wt. With this dietary regimen it was possible to obtain healthy animals of the same age with a vitamin A status that varied within a physiological range. LD₅₀-values for TCDD could not be calculated in any dietary group due to the unexpectedly low mortality rate. The obtained data, however, suggest that low dietary intake of vitamin A impairs the ability to tolerate the lethal effect of TCDD and/or decreases the survival time. Some TCDD-related effects, *i.e.* body weight loss, liver enlargement and decreased testes weight, were more pronounced in the groups receiving low levels of dietary vitamin A, whereas the decrease in thymus weight was as severe in all dietary groups. Exposure to TCDD had a pronounced effect on tissue vitamin A contents. The effect differed between tissues and was dependent upon the dose of TCDD and on the vitamin A status of the animal. Generally, exposure to TCDD results in the reduction of vitamin A in most organs; very likely because the capacity for vitamin A storage is diminished. The kidney is an exception because it accumulates vitamin A in response either to TCDD itself or to the altered vitamin A status which TCDD induces. Data presented in this study are not sufficient to conclude if the observed

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changes of tissue vitamin A contents are sufficient to explain the spectrum of morphological changes, which is typical of TCDD exposure.

Key Words 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD, vitamin A, diets

The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), on a molecular basis known to be the most toxic congener among the dibenzo-*p*-dioxins and dibenzofurans, interferes with the vitamin A turnover in the rat (1-10) and other species (11-13). These data suggest that TCDD interacts with the highly regulated but yet not fully understood process of vitamin A storage (14-16). Similarities of TCDD-induced symptoms and effects of dietary vitamin A deficiency in experimental animals are extensive, particularly as regards effects on growth regulation (17-19), cellular differentiation and proliferation (17,20), maintenance of epithelial tissues (17,20), and reproduction (17,21,22). The similarities include both the time-course for effect to occur and the quality of effect.

The present study was undertaken in order to investigate if dietary vitamin A would affect the toxicity of TCDD in the rat and if TCDD would affect the vitamin A homeostasis differently in animals with different vitamin A status.

MATERIALS AND METHODS

Animals and experimental design. Male Sprague-Dawley rats (ALAB, Solentuna, Sweden) in experimental groups of five TCDD-treated and four control animals, were fed purified casein based diets² stated to contain 2,000 (diet 1), 5,000 (diet 2), 8,000 (diet 3), or 21,000 (diet 4) IU of vitamin A/kg diet (Ewos AB, Södertälje, Sweden). Diets were stored at -20°C throughout the experiment. To obtain animals of the same age with variable vitamin A status at the time of TCDD-administration, feeding of experimental diets started immediately after weaning and continued throughout the study. After 5 weeks on the dietary regimens, experimental animals were given a single oral dose of 0, 15, 30, 60, or 120 µg TCDD/kg body wt. Two control rats from each dietary group were decapitated

² The raw materials were: Casein, corn starch, glucose, cellulose fiber, animal and vegetable fat, minerals, vitamin concentrate, and trace element concentrate. The composition was: 20% protein; 7% fat; 64% carbohydrate (corn starch: 44%, glucose: 20%); 4% fiber; 6% moisture. Vitamins per g diet were: vitamin A: retinylpalmitate as stated in the text; β-carotene: 0.1 µg; vitamin D: 1.5 IU; vitamin E: 42 µg; menadione: 1.5 µg; choline-chloride: 1 mg; thiamine: 4 µg; riboflavine: 12 µg; pyridoxine: 5 µg; vitamin B12: 40 ng; niacin: 40 µg; pantothenate: 10 µg. The minerals were: calcium: 0.6%; phosphorus: 0.3%; sodium: 0.3%; chloride: 0.5%; potassium: 0.5%; magnesium: 0.04%; iron: 292 mg/kg; manganese: 68 mg/kg; zinc: 7 mg/kg; copper 10 mg/kg; iodine: 2 mg/kg; cobalt: 0.4 mg/kg; selenium 100 µg/kg.

to provide data for tissue weights and vitamin A levels at the time of treatment. Animals were housed in wire bottomed plastic cages (350×250×180 mm). Illumination cycle, 12 h light and 12 h dark, was automatically controlled. Food and water were supplied *ad libitum*. Food consumption was not recorded. TCDD-treated animals were kept in a separate room in order to avoid any contamination of control animals and the surrounding laboratory. Body weights were recorded twice weekly. The surviving rats were killed by blood withdrawal under anesthesia (0.4 mg chloral hydrate in saline/kg body wt, i.p.) 44 days after treatment. Blood was centrifuged at 1,000×*g* and the collected serum was stored at -70°C. Tissues, i.e., liver, intestine, kidneys, testes, brain, lung, epididymis, thymus, spleen, and adrenals, were rapidly removed, rinsed in ice-cold saline and blotted with filter paper before weighing. Tissues were stored at -70°C until analyses were performed.

Chemicals. TCDD³ for administration (15, 30, 60, 120 µg/ml arachis oil) was prepared as previously described (3). All other chemicals were of analytical grade and commercially obtained.

Tissue extraction and vitamin A analysis. Completely hydrolyzed and extracted tissue vitamin A was analyzed as previously described (6).

Data analysis. Data reported are the arithmetic means for individual groups. Summary statistical analysis were performed by one- and two-way analysis of variance (ANOVA) using the SPSS/PC+TM statistical package (SPSS Inc.). The factor for pre-treatment data was diet. Classifiers for post-treatment data were dose and diet. One-way ANOVA and the Tukey-Kramer method for multiple comparisons (23) were used in order to identify significant differences among individual group means within each dietary group. Comparisons were considered significant at the $p < 0.05$ level. Data from animals that died prior to the termination of the study were excluded from the statistical analysis. Data from these animals are given as the arithmetic means ± standard deviation.

Safety precautions for the work with TCDD were as previously described (3).

RESULTS

Pre-treatment data

At the time of TCDD-treatment, i.e., after 5 weeks on the four different dietary regimens, tissues of two animals from each dietary group were analyzed to provide the base-line data on tissue weights and vitamin A contents. The different diets had no significant effects on body weight gain or on absolute and relative tissue weights (data not shown). There was a significant effect of diet on the vitamin A content in the liver, intestine, thymus, and adrenals (Tables 1 and 2). Less vitamin A seemed to be present in the testes, lung, spleen, and epididymis of animals in dietary

³ Lot no 351: 144-II, a generous gift from Dow Chemical Company, Midland, Michigan, U.S.A.

Table 1. The vitamin A content in tissues (μg) and serum ($\mu\text{g/ml}$) of male Sprague-Dawley rats fed diets containing 2,000, 5,000, 8,000 or 21,000 IU of vitamin A/kg for 5 weeks from weaning.^{1,2}

Diet (IU vitamin A/kg)	Vitamin A content in tissues (μg) and serum ($\mu\text{g/ml}$)									
	Liver	Intestine	Testes	Kidneys	Lung	Spleen	Epididymis	Thymus	Adrenals	Serum ³
2,000 (diet 1)	5	1.6	0.4	16.0	0.3	0.01	0.29	0.03	0.14	0.27
5,000 (diet 2)	148	3.8	1.2	8.3	1.7	0.03	0.60	0.06	0.23	0.35
8,000 (diet 3)	473	5.6	0.9	2.7	2.3	0.04	0.94	0.05	0.31	0.31
21,000 (diet 4)	1,020	6.4	1.0	3.0	3.8	0.04	0.84	0.06	0.48	0.40

¹Values are arithmetic means for 2 control rats from each dietary group. ²Summary statistical analysis were made by a one-way ANOVA where the factor was diet. Mean square values from the analysis of variance are presented in Table 3. ³The serum volume was estimated to be 3% of the body weight.

Table 2. Mean square values from the analysis of variance for parameter values presented in Table 1.

Source	D_f^1	Liver	Intestine	Testes	Kidneys	Lung	Spleen	Epididymis	Thymus	Adrenals	Serum
Diet	3	406,025***	9.0*	0.26	77	4.2	0.0003	0.17	0.0003**	0.041*	0.006
Residual	4	4,124	0.9	0.07	28	2.3	0.0001	0.06	0.0001	0.005	0.002

¹ D_b , degree of freedom. Statistically significant F -values are indicated by *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

group 1 as compared to animals in dietary groups 2, 3, and 4, while renal vitamin A, in contrast, was comparatively high in dietary groups 1 and 2 (Table 1). However, none of these differences were statistically significant (Table 2).

Post-treatment data

Non-surviving animals. Five animals in the group, which received diet 1, died from TCDD exposure; one at the 30 $\mu\text{g/kg}$ body wt dose level by day 37 after treatment and four at 120 $\mu\text{g/kg}$ body wt after 32 to 41 days. One animal at the highest dose, fed diet 2, died on day 31.

The mean body weight at autopsy for animals that died was 185 ± 32 g; corresponding to a body weight loss of 97 ± 33 g or $34 \pm 11\%$ of the body weight at the time of TCDD-treatment. The hepatic and intestinal vitamin A content was comparable in all animals that died, *i.e.*, about 2 μg in both tissues. In dietary group 2 testes, kidneys, lung, epididymis, and adrenals contained 0.2, 1.4, 0.2, 0.9, and 0.3 μg , respectively, whereas in dietary group 1 vitamin A was at or below the limit of detection in these tissues.

Surviving animals. Terminal body weight and body weight gain were comparable for control rats on the different diets (Table 3). For both parameters there was a pronounced TCDD-related decrease in all dietary groups (Tables 3 and 4). Animals, which received 120 μg TCDD/kg body wt, even lost weight as compared to the weight at the time of treatment (Tables 1 and 3). The loss of body weight was most pronounced in animals fed diets marginal (diet 2) or insufficient (diet 1) of vitamin A (Table 3). All organ weights except for the spleen were significantly affected by TCDD-treatment, while dietary vitamin A supplementation was without effect in all tissues but the intestine and adrenals (Table 4). In addition, a significant interaction between treatment and diet was found for several tissues (Table 4). Some TCDD-related effects *i.e.*, body weight loss, liver enlargement, and decreased testes weight, were more pronounced in the groups receiving low levels of dietary vitamin A, whereas the decrease in thymus weight was as severe in all dietary groups.

The vitamin A content of all tissues was significantly affected both by dose and diet (Tables 5 and 6). The two classifiers showed significant interaction for the vitamin A content in all tissues except for thymus (Table 6). Animals fed diet 1 had almost depleted liver stores of vitamin A, which could not be further reduced in response to TCDD-treatment (Table 5). In dietary group 2 there was an almost complete depletion of liver vitamin A already with the 15 $\mu\text{g/kg}$ body wt dose of TCDD, while 30 μg TCDD/kg body wt was needed for a comparable depletion in animals fed diet 3 (Table 5). The remaining vitamin A in the liver of animals fed diet 3 could not be depleted by the two higher doses of TCDD. Only in dietary group 4 was the TCDD-related hepatic vitamin A reduction linear over the whole dose-range (Table 5).

The intestinal vitamin A content of control animals was similar in all dietary groups (Table 5). Treatment with TCDD decreased the intestinal vitamin A

Table 3. Body weight (g), body weight gain (g) and tissue weights (g or mg) in male Sprague-Dawley rats 44 days after a single oral dose of 0, 15, 30, 60 or 120 μ g TCDD/kg body wt. Rats were maintained on diets containing 2,000, 5,000, 8,000 or 21,000 IU of vitamin A/kg from weaning throughout the experiment. TCDD was administered 5 weeks post-weaning.

Diet/dose	Body weight (g)	Body weight gain (g)	Tissue weights									
			Liver (g)	Intestine (g)	Testes (g)	Kidneys (g)	Brain (g)	Lung (g)	Spleen (g)	Epididymis (g)	Thymus (g)	Adrenals (mg)
2,000 IU vitamin A/kg												
0 μ g TCDD/kg bw	398	127	9.0	6.9	3.8	2.7	1.9	1.3	0.53	1.17	0.41	36
15	405	101	13.6 ^a	6.9	3.7	2.5	2.0	1.5	0.78	1.12	0.44	39
30	339	78	11.4	5.7	3.5	2.2	1.9	1.3	0.70	1.20	0.26	31
60	316	37 ^a	10.8	6.2	2.9	2.2	1.8	1.3	0.83	0.85	0.12	30
120	223 ^a	-59 ^a	15.8 ^a	6.2	0.7 ^a	2.0 ^a	2.0	1.7 ^a	1.46	0.42 ^a	0.04 ^a	51
5,000 IU vitamin A/kg												
0 μ g TCDD/kg bw	468	147	11.6	7.0	3.7	2.9	2.1	1.4	0.67	1.15	0.47	45
15	391 ^a	96 ^a	12.4	6.4	3.6	2.4	1.9 ^a	1.5	0.76	1.13	0.33	42
30	321 ^a	52 ^a	11.7	5.7	3.6	2.2 ^a	1.9 ^a	1.3	0.64	1.06	0.13 ^a	34
60	321 ^a	33 ^a	11.9	6.4	2.9	2.2 ^a	1.9 ^a	1.4	0.72	0.79 ^a	0.15 ^a	28 ^a
120	260 ^a	-33 ^a	14.4	5.8	1.2 ^a	2.2 ^a	1.8 ^a	1.2	0.79	0.38 ^a	0.03 ^a	34
8,000 IU vitamin A/kg												
0 μ g TCDD/kg bw	437	134	11.3	7.4	4.0	2.8	2.1	1.4	0.66	1.32	0.71	56
15	387	103	12.3	6.6	3.6	2.3 ^a	1.8 ^a	1.5	0.79	1.14	0.40 ^a	44
30	373	78	14.0	6.6	3.5	2.6	1.9	1.4	0.82	1.14	0.28 ^a	40
60	322 ^a	49 ^a	13.1	5.6	3.0	2.2 ^a	1.8 ^a	1.3	0.69	0.83 ^a	0.09 ^a	39 ^a
120	281 ^a	-4 ^a	12.9	5.5	2.2 ^a	2.0 ^a	1.8 ^a	1.3	0.82	0.61 ^a	0.06 ^a	40
21,000 IU vitamin A/kg												
0 μ g TCDD/kg bw	432	136	10.8	7.4	4.1	2.9	2.0	1.4	0.91	1.24	0.54	53
15	359	77	11.0	6.7	3.5	2.2 ^a	2.0	1.5	0.66	1.08	0.37	40
30	368	96	11.8	7.0	3.5	2.4	1.9	1.6	0.92	1.09	0.36	42
60	345 ^a	57 ^a	12.8	6.7	3.1	2.5	1.9	1.5	0.84	0.94	0.09 ^a	32
120	298 ^a	2 ^a	13.7	7.0	2.6	2.1 ^a	1.8	1.3	0.77	0.72 ^a	0.06 ^a	34

¹ Values are arithmetic means for 2 control or 5 TCDD-treated rats except for the groups of rats fed diet 1 that received 30 μ g TCDD/kg body wt ($n=4$) and 120 μ g TCDD/kg body wt ($n=1$) and the group of rats fed diet 2 that received 120 μ g TCDD/kg body wt ($n=4$). ² Summary statistical analyses were made by a two-way ANOVA where the factors were dose and diet. Mean square values from the analysis of variance are presented in Table 4. ^a Indicate significant differences ($p<0.05$) when compared to corresponding controls within the same dietary group.

Table 4. Mean square values from the analysis of variance for parameter values presented in Table 3.

Source	D_f^1	Body weight	Body weight gain	Liver	Intestine	Testes	Kidneys	Brain	Lung	Spleen	Epididymis	Thymus	Adrenals
Dose	4	43,373***	40,015***	12.4*	2.15*	8.28***	0.72***	0.08***	0.10**	0.03	1.00***	0.49***	431***
Diet	3	1,257	1,455	2.7	2.50*	0.53	0.02	0.01	0.04	0.03	0.05	0.02	233**
Dose $\times$ diet	12	2,011	835	5.4	0.78	0.48	0.10*	0.02	0.04*	0.07***	0.02	0.02**	75
Residual	62	1,158	718	3.9	0.63	0.42	0.04	0.01	0.02	0.02	0.03	0.01	50

¹ D_f , degree of freedom. Statistically significant F -values are indicated by *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 5. The vitamin A content in tissues (μg) and serum ($\mu\text{g}/\text{ml}$) of male Sprague-Dawley rats 44 days after a single oral dose of 0, 15, 30, 60 or 120 μg TCDD/kg body wt. Rats were maintained on diets containing 2,000, 5,000, 8,000 or 21,000 IU of vitamin A/kg from weaning throughout the experiment. TCDD was administered 5 weeks post-weaning.^{1,2}

Diet/dose	Vitamin A content in tissues (μg) and serum ($\mu\text{g}/\text{ml}$)									
	Liver	Intestine	Testes	Kidneys	Lung ³	Spleen ³	Epididymis	Thymus ³	Adrenals	Serum ⁴
2,000 IU vitamin A/kg										
0 μg TCDD/kg bw	0.59	3.4	0.1	1.9			0.34		0.12	0.11
15	0.46	0.3 ^a	0.2	0.9 ^a			0.13		0.07	0.09
30	0.40	0.2 ^a	0.1	0.6 ^a			0.08		0.02	0.10
60	0.44	0.2 ^a	0.1	0.6 ^a			0.04		0.04	0.08
120	0.43	0.3 ^a	ND	ND			ND		0.004 ^a	0.01
5,000 IU vitamin A/kg										
0 μg TCDD/kg bw	184.4	4.4	0.9	14.9	0.7	0.04	1.4	0.07	0.65	0.38
15	1.5 ^a	1.7 ^a	0.6	12.5	0.3 ^a	0.21 ^a	0.7 ^a	0.40	0.39	0.23
30	1.1 ^a	1.6 ^a	0.6 ^a	6.3	0.2 ^a	0.17 ^a	0.6 ^a	0.30	0.27 ^a	0.17 ^a
60	0.5 ^a	1.5 ^a	0.5 ^a	3.1 ^a	0.2 ^a	0.14 ^a	0.5 ^a	0.36	0.23 ^a	0.18 ^a
120	0.5 ^a	1.4 ^a	0.3 ^a	1.3 ^a	0.2 ^a	0.14 ^a	0.3 ^a	0.30	0.39	0.06 ^a
8,000 IU vitamin A/kg										
0 μg TCDD/kg bw	740	3.6	1.0	2.5	0.6	0.05	1.7	0.06	1.04	0.40
15	90 ^a	4.0	0.8	9.1	1.4	0.19 ^a	1.8	0.04	0.80	0.45
30	16 ^a	3.7	0.9	10.0 ^a	1.2	0.16 ^a	1.6	0.04	0.61	0.37
60	22 ^a	2.3	0.7	5.6	0.7	0.13	1.6	0.01	0.70	0.26
120	11 ^a	2.0	0.4	1.5	0.4	0.12	1.0	0.01	0.48 ^a	0.21
21,000 IU vitamin A/kg										
0 μg TCDD/kg bw	2,235	3.4	0.6	1.9	2.1	0.06	0.8	0.06	1.16	0.36
15	907 ^a	8.1 ^a	2.7 ^a	7.5	0.2 ^a	0.22	4.2 ^a	0.16	2.43 ^a	0.44
30	500 ^a	8.8 ^a	2.7 ^a	10.6 ^a	0.4 ^a	0.30 ^a	4.4 ^a	0.16	2.34	0.42
60	417 ^a	8.9 ^a	2.5 ^a	8.6 ^a	0.1 ^a	0.27 ^a	3.3 ^a	0.15	1.53	0.56
120	229 ^a	7.4 ^a	2.1	6.0	0.1 ^a	0.27 ^a	2.7 ^a	0.13	1.60	0.53

¹Values are arithmetic means for 2 control or 5 TCDD-treated rats except for the groups of rats fed diet 1 that received 30 μg TCDD/kg body wt ($n=4$) and 120 μg TCDD/kg body wt ($n=1$) and the group of rats fed diet 2 that received 120 μg TCDD/kg body wt ($n=4$). ²Summary statistical analyses were made by a two-way ANOVA where the factors were dose and diet. Mean square values from the analysis of variance are presented in Table 6. ³The analyses of vitamin A for lung, spleen, and thymus were made at or near the limit of detection (about 2 ng of retinol) for most individuals in dietary group 1. Therefore this data is lacking in Tables 3 and 4. ⁴The serum volume was estimated to be 3% of the body weight. ^aIndicates a significant difference ($p<0.05$) when compared to corresponding controls. ND=non detectable.

Table 6. Mean square values from the analysis of variance for parameter values presented in Table 5.

Source	D_f^1	Liver ²	Intestine	Testes ²	Kidneys	Lung ³	Spleen ⁴	Epididymis ⁵	Thymus ⁶	Adrenals ⁵	Serum ⁵
Dose	4	912,702***	2.3*	0.64**	93.2***	1.04***	0.031***	2.0***	0.03*	0.34**	0.02*
Diet	3	2,257,716***	210.3***	18.58***	210.3***	2.53***	0.073***	41.8***	0.43***	13.49***	0.58***
Dose × diet	12	329,506***	7.4***	0.61***	45.4***	0.77***	0.006*	1.8***	0.01	0.34***	0.03**
Residual	62	14,515	0.8	0.16	9.1	0.08	0.003	0.1	0.01	0.08	0.01

¹ D_f , degree of freedom. Statistically significant F -values are indicated by *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. ² d_f for the residual was 60. ³ d_f for the sources dose, diet, dose × diet and residual were 4, 2, 8 and 49, respectively. ⁴ d_f for the sources dose, diet, dose × diet and residual were 4, 2, 8 and 50, respectively. ⁵ d_f for the residual was 61. ⁶ d_f for the sources dose, diet, dose × diet and residual were 4, 2, 8 and 44, respectively.

content in dietary groups 1, 2, and 3, but increased it in group 4 (Tables 5 and 6). Testes and epididymis of control animals contained about 1 μg vitamin A in all but dietary group 1, in which lower levels were noted (Table 5). In dietary group 1, TCDD-exposure resulted in decreasing levels of vitamin A in epididymis, whereas no effect was observed in testes (Table 5). Both tissues showed decreasing levels of vitamin A as a result of TCDD-treatment in dietary group 2, while no effect and an increase, respectively, was observed in dietary groups 3 and 4; the higher the dose of TCDD the less pronounced the increase (Table 5).

Control levels of vitamin A in the kidneys were similar in all but dietary group 2, where considerably more vitamin A was found (Table 5). TCDD-treatment decreased the renal vitamin A content in dietary groups 1 and 2, but increased it above control levels in dietary groups 3 and 4 (Table 5). The TCDD-induced increase in renal vitamin A became less pronounced with the dose both in dietary groups 3 and 4 (Table 5).

Increasing dietary vitamin A intake resulted in increased vitamin A content of control lungs and adrenals but had no effect on the vitamin A content in spleen and thymus of control animals (Table 5). TCDD-treatment decreased the vitamin A content in the lung of rats in dietary groups 2 and 4, while no consistent effect was observed in dietary group 3 (Table 5). The spleen, in contrast, showed increased vitamin A content as a consequence of TCDD-injection in all three dietary groups (Table 5). Thymic vitamin A was increased in TCDD-exposed rats fed diets 2 and 4, while no effect was present in dietary group 3 (Table 5). Decreasing adrenal vitamin A was observed in TCDD-treated animals fed diets 1, 2, and 3, while animals fed diet 4 showed increased adrenal vitamin A, more so with the lower doses than with the higher (Table 5).

Serum vitamin A was comparable in control rats fed diets 2, 3, and 4, whereas about 70% lower serum values were noticed for animals fed diet 1 (Table 5). TCDD-exposure resulted in decreasing serum vitamin A levels in rats from dietary groups 1, 2, and 3, while a tendency towards increasing serum vitamin A was observed in dietary group 4 (Table 5).

DISCUSSION

The survival was unexpectedly high in the present study as compared to the previously published i.p. LD_{50} -value of 60 μg TCDD/kg body wt in adult male Sprague-Dawley rats (24). LD_{50} -values well above 120 $\mu\text{g}/\text{kg}$ body wt, however, have been reported for adult Fisher 334N, CD, and Han/Wistar rats given TCDD orally in corn oil (25,26). Due to the low number of dead animals in this study it was not possible to calculate LD_{50} -values for TCDD in any dietary group. The present data, however, suggest that a lower dose of TCDD will be needed to cause death in animals fed a diet containing 5,000 IU of vitamin A/kg, or less. The data also suggest that the LD_{50} -value for animals receiving 2,000 IU of vitamin A/kg diet is in the range 60–120 μg TCDD/kg body wt, and above 120 μg TCDD/kg body wt

for animals that receive 5,000 IU of vitamin A/kg diet, or more. Thus, it seems that low dietary intake of vitamin A impairs the ability to tolerate the lethal effect of TCDD, and/or at least decreases the survival time.

A factor other than route of administration, vehicle and strain of rat used, which may contribute to the obtained lethality, is the use of a diet based on casein. There are several studies suggesting that ordinary cereal-based animal feed may increase the toxic effect, using preneoplastic changes in the liver as end-point, as compared to casein-based diets (27-30). The reason for these differences is not clear at present, but it cannot be excluded that the use of a purified diet in this study may have contributed to the low mortality rate observed.

Decreased body weight gain is a major symptom of both TCDD-exposure (17) and dietary vitamin A deficiency (18,19), thus it was of interest to compare changes in tissue-to-body weight ratios obtained in this study, with changes observed in cases of non-toxic growth reduction (31-33) and/or dietary vitamin A deficiency (34). Based on such comparisons, it appears that the observed increases in liver-, kidney-, and spleen-to-body weight ratios (data from Table 3) are separate both from growth reduction per se as well as from vitamin A deficiency, while the increase in brain-to-body weight ratio seen in TCDD-exposed animals (data from Table 3) most likely is secondary to the reduced body weight gain. It cannot be deduced from data in this (Table 3) and other studies (31-34) whether the reduced testes-, epidymidis-, and thymus-to-body weight ratios and the increased lung- and intestine-to-body weight ratios are primary effects of TCDD-exposure, vitamin A deficiency, and/or of both.

With the dietary regimens used in the present study it was possible to obtain healthy animals of the same age with a vitamin A status that varied within a physiological range. Within the 5 pre-treatment weeks, diet 1 produced moderately vitamin A-deficient animals as judged by normal body weight gain, almost depleted hepatic stores of vitamin A, increased renal vitamin A, and normal serum vitamin A (Table 1). At the end of the study, control animals fed diet 1 were depleted of hepatic vitamin A, serum vitamin A was below normal, and renal vitamin A was low as compared to 44 days earlier (Table 5). However, body weight gain and relative tissue weights were not different from other dietary groups (Table 3). Diet 2 produced control animals which were marginally vitamin A deficient throughout the study. Rats in this group were able to maintain both the hepatic and serum vitamin A levels (Tables 1 and 5). Renal vitamin A, which was high at the time of treatment, became even higher and approached a value comparable that found in pre-treatment control rats fed diet 1 at the end of the study (Tables 1 and 5). Control animals fed diets 3 and 4 were able to increase the hepatic vitamin A content and to maintain the renal and serum vitamin A levels at similarly low levels throughout the study (Tables 1 and 5). Vitamin A contents in other extrahepatic tissues of control animals remained constant throughout the study (Tables 1 and 5).

Due to the fact that TCDD-reduced food intake is dose-dependent (17), it is unfortunate that no attempts were made to measure food consumption in this

study. However, previous studies have shown that the TCDD-induced reduction of hepatic vitamin A cannot be fully explained by reduced food intake (9, 10, 35, 36).

The liver plays a central role in the turnover, metabolism and storage of vitamin A in the body. The parenchymal cells, which constitute the main part of the liver, play a major role in the uptake of vitamin A from serum, while stellate cells are responsible for the main part of hepatic vitamin A storage (14–16). It is known from previous studies that TCDD reduces the hepatic accumulation of ingested vitamin A (3) and increases its mobilization from liver reserves (5). The decrease mainly concerns the storage form of vitamin A, *i.e.*, the retinylesters (2), which are reduced both in liver parenchymal cells and stellate cells (13). It is possible, but not very likely, that TCDD-induced reduction in intestinal absorption and/or hepatic internalization of vitamin A can account for the reduction in hepatic vitamin A content (37). In the present study, exposure to TCDD resulted in a marked decrease in the hepatic vitamin A content as compared to the control level, both at the time of exposure and at termination in all but dietary group 1 (Tables 1 and 5). Increasing the doses of TCDD was required in order to deplete hepatic stores of vitamin A in dietary groups 2, 3, and 4, respectively, while diet 1 in itself depleted liver stores to a minimum level (Table 5). The “undepletable” amount of hepatic vitamin A in dietary group 3 was higher than in dietary groups 1 and 2, and more hepatic vitamin A (absolute amount) was depleted with the same dose of TCDD in rats with high as opposed to low liver reserves (Table 5). Since the amount of vitamin A in stellate cells increases markedly as a result of increased dietary vitamin A intake, while the amount of vitamin A in parenchymal cells remains more or less the same (14), our data support the notion that TCDD primarily damages the vitamin A-storing capacity of the stellate cells. A decrease in the vitamin A content in stellate cells would occur if TCDD inhibits the transfer of vitamin A from the parenchymal cell into the stellate cell and/or triggers the release of vitamin A from the stellate cell into the circulation. Further studies are needed to explore these possibilities. Damage could also be a consequence of cytotoxicity and/or transformation of the stellate cell into a cell lacking the capacity to house vitamin A. Although the liver is a well-known target organ for TCDD (17), its effect on the number, morphology and/or function of stellate cells has not been investigated. A related but less toxic and persistent compound, 3,3',4,4'-tetrachlorobiphenyl, however, produces minimal morphological changes in the stellate cells at doses where alterations in hepatic vitamin A content and parenchymal cell morphology are observed (38).

The comparatively high levels of vitamin A in serum and several peripheral tissues observed in rats with concurrent high vitamin A status and high TCDD-exposure (Table 5), are likely to occur because vitamin A overflows the hepatic storage capacity in cases of severe liver damage. A linear dose-response relationship for hepatic vitamin A reduction was present in dietary group 4 only (Table 5). Since inhibited hepatic vitamin A accumulation tends to last longer in animals receiving a high, compared with a low, dose of TCDD (11), one may speculate that

a linear effect over the whole dose-range might also have developed in dietary groups 2 and 3, had the study lasted long enough to allow animals from all groups to regain their full capacity to store vitamin A.

Unaffected, increased, and decreased levels of serum vitamin A have been recorded in rats of variable vitamin A status exposed to single doses of TCDD. Increases in serum vitamin A levels were found in adult (1,3,6) and weanling-aged (11) vitamin A sufficient rats, while no effect or decreases were found in deficient or marginally vitamin A sufficient adult rats (2,5,27). These observations are in agreement with results in the present study (Table 5). In general, the response, which among other things seems to depend on dose, time after treatment, and hepatic vitamin A reserves, was small and variable. Only animals with depleted liver reserves showed serum levels of vitamin A characteristic of vitamin A deficiency (Table 5). The most pronounced increase in serum vitamin A was observed in rats receiving diet 4 and the two high doses of TCDD (Table 5). This elevation would indicate that severe liver damage contributes to the increased level of circulating vitamin A. It is known that plasma levels of retinol are regulated within narrow homeostatic limits independent of alterations in liver reserves over a broad range. Only when the reserves are critically depleted or excessively saturated is there a change in plasma levels. Control mechanisms for maintaining the homeostatic level of circulating retinol are not known in detail but seem to involve both the generation of extrahepatic regulatory signals during the uptake and/or utilization of retinol in target tissues (39,40), the hepatic synthesis and the secretion of the retinol-binding protein (41). The lack of an immediate and pronounced effect of TCDD on the serum vitamin A level in this and previous studies makes it unlikely that TCDD has a direct effect on the proposed homeostatic control of the vitamin A blood level.

The effect of TCDD on renal vitamin A differed markedly depending on the dose of TCDD and on the vitamin A status of the animal (Table 5). A pronounced increase in renal levels of vitamin A following TCDD treatment was observed in vitamin A sufficient rats, despite the fact that these animals still had considerable hepatic stores of vitamin A, and continued to eat the same vitamin A-containing diet (Table 5). In contrast, decreasing renal vitamin A levels were observed in rats which, due to poor vitamin A supplementation and/or TCDD-exposure, had marginal or depleted liver reserves (Table 5). The accumulation of vitamin A in the kidney was maximal at doses of 15–30 μg TCDD/kg body weight, while decreasing levels were found at higher doses (Table 5). These data are in agreement with those of Jurek *et al.* (42), who also demonstrated that both exposure to TCDD and intake of a vitamin A-free diet induce a concurrent increase in the renal formation of retinylesters. Taken together, these data suggest that TCDD induces a condition that mimics vitamin A deficiency with respect to renal vitamin A accumulation (42–44). Since the kidney is one of the few organs more or less resistant to the toxicity of TCDD (17), it is unlikely that the response obtained is due to TCDD-induced damage of the kidney. The present and previous

(1-3, 5-8, 12, 42) results, rather, suggest that TCDD treatment disturbs the mechanism for monitoring changes in liver reserves and/or exogenous supply of vitamin A, which has been proposed to be localized to the kidney (43, 44).

Vitamin A contents in the intestine, testes, lung, spleen, epididymis, thymus, and adrenal glands of control rats receiving diets 2, 3, and 4 were roughly comparable for each individual organ (Table 5). This would suggest that peripheral tissue contents of vitamin A are maintained fairly constant over a broad range of liver reserves and dietary vitamin A intakes. In contrast, inadequate vitamin A intake (diet 1) results in markedly lower peripheral tissue levels (Table 5). The effect of TCDD on peripheral tissue levels of vitamin A in the rat varied markedly with the dietary vitamin A status of the animals. Rats receiving diets containing low and intermediate levels of vitamin A generally showed decreased or unaffected vitamin A contents in peripheral tissues as a result of TCDD exposure, while tissues of rats fed excess amounts of vitamin A generally showed increased vitamin A contents (Table 5). Decreases in tissue vitamin A levels induced by TCDD may be the result of decreased storage (similar to the effect in the liver), or increased utilization of vitamin A, which is not compensated for by increased vitamin A distribution to that particular tissue. The increased tissue levels of vitamin A, observed in TCDD exposed animals having large liver reserves and continued excessive intake of vitamin A, may be a consequence of increased amounts of circulating vitamin A. This vitamin may either be preferentially stored in extrahepatic tissues or retained there because it cannot be eliminated; possibly because the system for "non-functional" metabolism of vitamin A is overloaded.

To summarize, data presented herein show that dietary vitamin A provides limited protection against some typical TCDD-related toxic effects. Exposure to TCDD has a pronounced effect on tissue vitamin A contents. The effect differs between tissues and is dependent upon the dose of TCDD and the vitamin A status of the animal. Generally, exposure to TCDD results in the reduction of vitamin A in most organs; most likely because the capacity for vitamin A storage is diminished. The kidney is an exception, because it accumulates vitamin A either in response to TCDD itself or to the altered vitamin A status which it induces. The pronounced effect of TCDD on tissue vitamin A contents, which differs between tissues, is markedly dependent upon the vitamin A status of the animal. The tissue level of vitamin A required to maintain proper tissue histology and function *in vivo* is not known for any target organ. Thus, conclusions as to whether the observed changes of tissue vitamin A levels are responsible for the spectrum of morphological changes observed after TCDD exposure cannot be drawn, based on data presented in this study. However, it is likely that decreasing levels of vitamin A in target tissues adversely affects the functioning of these organs. On the other hand, increasing levels of vitamin A are more likely to be harmless, at least in tissues capable of storing excess vitamin A.

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