

per cage, and dosed with TCDD or corn oil. The animals were kept in temperature- and humidity-controlled rooms with a 12-hour light/dark cycle. The access to animal rooms was limited and kept to a minimum.

TCDD solution for dosing these animals was prepared by dissolving 5 mg of the chemical (> 99% purity) initially into 10 ml acetone (glass distilled) and mixing this solution with 190 ml of corn oil (Nutritional Biochemicals, Cleveland, Ohio). This stock solution, containing 25 µg TCDD/ml was diluted with corn oil containing 5% acetone to provide appropriate dosing solutions. The control group was dosed with corn oil containing 5% acetone. The concentrations of all dosing solutions were appropriate to provide 0.1 ml/10 g to mice. The animals were weighed and dosed once a week with 0.0, 0.01, 0.10, 1.0, and 10 µg/kg of TCDD, for 8 weeks. In addition, one group of mice were given a single oral dose of TCDD at 10 µg/kg body weight.

Four animals were randomly selected from each group and sacrificed at 2, 4, and 8 weeks after the treatment started. Similarly, the animals were killed 2, 4, and 8 weeks after a single treatment with TCDD. At the time of sacrifice, the blood was saved for serum and selected organs were collected and weighed. The liver, kidney, and thymus were placed in formalin while the spleen was saved in sterilized saline for lymphocyte cultures.

The tissues from these animals were routinely processed and stained for histopathologic evaluation. Microscopic examination of all organs was performed in a random-blind fashion.

#### *Splenic Lymphocyte Cultures*

Immediately after their collection, the spleens were washed with sterilized isotonic saline solution and mashed with a pair of forceps in saline to release their cellular constituents. The cells were separated from the intact tissue by serially passing through 18, 21, and 26 gauge needles. The solution was allowed to stand in a glass tube for nearly 10 minutes to remove cell aggregates, after which it was transferred to a different tube leaving the sediment and glass adherent materials behind. This suspension was centrifuged at 4000 rpm for 10 minutes at room temperature and the cell pellet suspended in a small volume (3-5 ml) of culture medium. An aliquot was used for counting cells in an electronic counter after lysis of erythrocytes.

Lymphocyte transformation was determined in cultures by a method essentially modified from Alhaji *et al.*<sup>6</sup> The culture media used was RPMI 1640 (Grand Island Biologicals, Grand Island, N.Y.) containing 10% heat-inactivated fetal calf serum and 100 units of penicillin and 100 µg of streptomycin per ml. The media was supplemented with 300 µg/ml of L-glutamine. The cell suspension was adjusted to provide approximately  $8 \times 10^6$  cells/ml and 50 µl of this layered into each well of a micro-tissue-culture plate (Microtest II, Falcon 3040, Falcon Plastic, Oxnard, Ca). To each well, 50 µl of additional culture media was added and 50 µl of media either without any mitogen or containing optimized concentrations of phytohemagglutinin (PHA, 1 µl, reconstituted, per culture well) or pokeweed mitogen (PWM, 1 µl, per culture well, both mitogens obtained from Grand Island Biologicals, Grand Island, N.Y.). The plates were incubated for 52 hours at 37° C in humidified 5% CO<sub>2</sub> in air atmosphere. After this incubation, 50 µl of culture media containing 0.5 µCi of <sup>3</sup>H-thymidine (New England Nuclear, Boston, Mass.) was added to each culture well and the incubation continued for an additional 16 hours. The cells were harvested and vigorously washed with physiologic saline using a model M12V Cell Harvester (Brandel, Co., Rockville, Md.) and collected on Reeve Angel 934AH glass fiber discs (Whatman, Inc., Clifton, N.J.). These discs were placed directly in regular counting

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vials, 10 ml of scintillation solution (AquaSol, New England Nuclear, Boston, Mass.) added and the radioactivity counted after shaking in a Mark III Scintillation spectrometer (Searle Analytix, Inc., Chicago, Ill.). The counts were converted to disintegrations per minute (DPM) using a standard quench curve, averaged and adjusted for 10<sup>6</sup> cells in the culture system. All cultures were run in triplicate. The stimulation index for PHA or PWM was calculated by dividing the DPM for cultures in the presence of respective mitogen by the DPM for cultures in which no mitogen was present.

To determine the effect of TCDD directly on lymphocytes in culture, the spleens were collected from control animals and cultured in media containing various concentrations of TCDD (see RESULTS section) as described above.

#### Estimation of Immunoglobulins

Total immunoglobulins in sera of animals were estimated by a quantitative immunoelectrophoresis (QIEF) technique modified from Bjerrum *et al.*<sup>7</sup> Rabbit anti-mouse Ig. globulin fraction (BioRad Laboratories, Richmond, CA), was carbamylated and incorporated into a 1% agarose gel plate (10 cm X 10 cm X 1.5 mm). Appropriately diluted serum samples were applied in duplicate in wells punched into gel plates containing respective antiglobulin and electrophoresis performed (300 volts for 3-4 hours or 100 volts for 16 hours) using a Model 1400 electrophoresis chamber (BioRad Laboratories, Richmond, CA). The plates were pressed, dried and stained with coomassie brilliant blue, and the heights of rockets compared to appropriate standard samples in the same plate. A pooled mice serum sample (pooled from juvenile male animals) was used as a reference standard and all Ig concentrations represented as a ratio to this pooled sample.

#### Statistical Analysis

The values are represented as means and standard deviation or standard error of means. The parameters of treatment group were compared to those in the respective control group by using a t-test. In some cases, two-way analysis of variance was performed. The body weights, organ weights and their ratios were compared using a Dunnett's 't'.<sup>8</sup>

#### RESULTS

TCDD produced toxicologic manifestations in mice at high levels of exposure. Their body and organ weights, at different periods during the experimentation, are given in TABLE 1. As indicated, the increase in the body weight of mice exposed to 10  $\mu$ g/kg/wk TCDD for 8 weeks was due to the accumulation of ascitic fluid (animals with severe ascites were not sampled). The only definitive changes in organ weights were increased liver weights in mice receiving 10  $\mu$ g TCDD/kg/week.

Histopathologic examination of organs showed consistent treatment-related changes only in mouse livers. The hepatic changes were similar to those described previously for rats.<sup>9</sup> The lesions ranged from swelling and vacuolation of hepatocytes to autolysis and necrosis (both focal and diffuse) of parenchymal tissue. The lesions were primarily in peripheral lobular regions and in some cases nearly  $\frac{1}{2}$  of the total tissue indicated extensive necrosis. Some sections also exhibited fibrosis, increased

TABLE I  
ORGAN AND BODY WEIGHTS OF MICE TREATED WITH TCDD FOR DIFFERENT INTERVALS\*

| Duration of Exposure | Exposure Level<br>$\mu\text{g/kg/week}$ | Body Weight<br>(g) | Organ Weights and Their Relation to Body Weight |                 |                 |                 |                 |                 |                 |                 |
|----------------------|-----------------------------------------|--------------------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                      |                                         |                    | Thymus                                          |                 | Spleen          |                 | Liver           |                 | Kidney          |                 |
|                      |                                         |                    | (g)                                             | (g/100g)        | (g)             | (g/100g)        | (g)             | (g/100)         | (g)             | (g/100g)        |
| Precapexure          | 0                                       | 28 $\pm$ 4         | 0.05 $\pm$ 0.01                                 | 0.18 $\pm$ 0.02 | 0.09 $\pm$ 0.02 | 0.31 $\pm$ 0.06 | 1.67 $\pm$ 0.24 | 6.06 $\pm$ 0.35 | 0.48 $\pm$ 0.07 | 1.75 $\pm$ 0.09 |
| 2 weeks              | 0                                       | 33 $\pm$ 1         | 0.07 $\pm$ 0.01                                 | 0.20 $\pm$ 0.03 | 0.07 $\pm$ 0.01 | 0.20 $\pm$ 0.04 | 1.90 $\pm$ 0.17 | 5.79 $\pm$ 0.42 | 0.55 $\pm$ 0.05 | 1.69 $\pm$ 0.03 |
|                      | 0.01                                    | 36 $\pm$ 1         | 0.06 $\pm$ 0.01                                 | 0.17 $\pm$ 0.03 | 0.08 $\pm$ 0.01 | 0.23 $\pm$ 0.03 | 1.99 $\pm$ 0.11 | 5.53 $\pm$ 0.32 | 0.56 $\pm$ 0.05 | 1.55 $\pm$ 0.15 |
|                      | 0.1                                     | 36 $\pm$ 2         | 0.07 $\pm$ 0.01                                 | 0.18 $\pm$ 0.02 | 0.10 $\pm$ 0.02 | 0.28 $\pm$ 0.05 | 2.12 $\pm$ 0.20 | 5.87 $\pm$ 0.21 | 0.55 $\pm$ 0.06 | 1.52 $\pm$ 0.15 |
|                      | 1                                       | 35 $\pm$ 2         | 0.05 $\pm$ 0.01                                 | 0.13 $\pm$ 0.01 | 0.10 $\pm$ 0.01 | 0.27 $\pm$ 0.03 | 2.24 $\pm$ 0.15 | 6.36 $\pm$ 0.39 | 0.64 $\pm$ 0.06 | 1.81 $\pm$ 0.10 |
|                      | 10                                      | 35 $\pm$ 2         | 0.06 $\pm$ 0.01                                 | 0.16 $\pm$ 0.01 | 0.09 $\pm$ 0.02 | 0.26 $\pm$ 0.06 | 2.77 $\pm$ 0.32 | 7.83 $\pm$ 0.57 | 0.57 $\pm$ 0.08 | 1.61 $\pm$ 0.17 |
| 4 weeks              | 0                                       | 38 $\pm$ 3         | 0.06 $\pm$ 0.02                                 | 0.16 $\pm$ 0.04 | 0.09 $\pm$ 0.03 | 0.25 $\pm$ 0.05 | 1.94 $\pm$ 0.32 | 5.14 $\pm$ 0.53 | 0.55 $\pm$ 0.07 | 1.45 $\pm$ 0.09 |
|                      | 0.01                                    | 39 $\pm$ 3         | 0.04 $\pm$ 0.02                                 | 0.11 $\pm$ 0.05 | 0.09 $\pm$ 0.02 | 0.24 $\pm$ 0.03 | 1.91 $\pm$ 0.20 | 4.92 $\pm$ 0.38 | 0.58 $\pm$ 0.07 | 1.48 $\pm$ 0.10 |
|                      | 0.1                                     | 39 $\pm$ 3         | 0.04 $\pm$ 0.01                                 | 0.10 $\pm$ 0.03 | 0.07 $\pm$ 0.02 | 0.19 $\pm$ 0.03 | 2.00 $\pm$ 0.24 | 5.16 $\pm$ 0.46 | 0.58 $\pm$ 0.07 | 1.48 $\pm$ 0.16 |
|                      | 1                                       | 38 $\pm$ 5         | 0.05 $\pm$ 0.01                                 | 0.13 $\pm$ 0.03 | 0.09 $\pm$ 0.02 | 0.24 $\pm$ 0.06 | 2.32 $\pm$ 0.34 | 6.11 $\pm$ 0.90 | 0.61 $\pm$ 0.10 | 1.59 $\pm$ 0.17 |
|                      | 10                                      | 37 $\pm$ 2         | 0.04 $\pm$ 0.01                                 | 0.08 $\pm$ 0.04 | 0.11 $\pm$ 0.04 | 0.30 $\pm$ 0.10 | 2.79 $\pm$ 0.53 | 7.54 $\pm$ 0.94 | 0.62 $\pm$ 0.11 | 1.68 $\pm$ 0.24 |
| 8 weeks              | 0                                       | 40 $\pm$ 1         | 0.05 $\pm$ 0.01                                 | 0.13 $\pm$ 0.01 | 0.09 $\pm$ 0.01 | 0.23 $\pm$ 0.04 | 1.96 $\pm$ 0.19 | 4.92 $\pm$ 0.43 | 0.57 $\pm$ 0.02 | 1.43 $\pm$ 0.07 |
|                      | 0.01                                    | 39 $\pm$ 4         | 0.06 $\pm$ 0.02                                 | 0.16 $\pm$ 0.05 | 0.13 $\pm$ 0.10 | 0.34 $\pm$ 0.29 | 2.03 $\pm$ 0.16 | 5.19 $\pm$ 0.27 | 0.54 $\pm$ 0.09 | 1.38 $\pm$ 0.23 |
|                      | 0.1                                     | 42 $\pm$ 3         | 0.07 $\pm$ 0.03                                 | 0.16 $\pm$ 0.07 | 0.11 $\pm$ 0.04 | 0.26 $\pm$ 0.12 | 2.30 $\pm$ 0.17 | 5.50 $\pm$ 0.08 | 0.60 $\pm$ 0.07 | 1.43 $\pm$ 0.20 |
|                      | 1                                       | 44 $\pm$ 4         | 0.07 $\pm$ 0.01                                 | 0.16 $\pm$ 0.04 | 0.11 $\pm$ 0.02 | 0.25 $\pm$ 0.05 | 2.83 $\pm$ 0.22 | 6.49 $\pm$ 0.41 | 0.60 $\pm$ 0.07 | 1.36 $\pm$ 0.13 |
|                      | 10                                      | 49 $\pm$ 3         | 0.05 $\pm$ 0.01                                 | 0.11 $\pm$ 0.02 | 0.11 $\pm$ 0.03 | 0.23 $\pm$ 0.06 | 3.28 $\pm$ 0.36 | 6.75 $\pm$ 0.46 | 0.56 $\pm$ 0.03 | 1.16 $\pm$ 0.11 |

\* Mean  $\pm$  S.D. of 4 animals per group, except as indicated.

† Only 3 animals in this group.

‡ Indicate significant deviation from control by Dunnett's test ( $p < .05$ ). The increased body weight in the last group in the table (8 week exposure, 10  $\mu\text{g/kg/week}$ ) was considered to be due to the presence of ascitic fluid.

leukocytic infiltration and perivascular degeneration and necrotic changes. The incidence of liver and thymus lesions in mice exposed to various levels of TCDD for different durations is shown in TABLE 2. The lesions observed in control and 0.01  $\mu\text{g/kg/wk}$  TCDD-exposed group were minor.

Treatment-related lesions of the thymus were noted only after 4 weeks of exposure in groups given 1 or 10  $\mu\text{g TCDD/kg/week}$  (TABLE 2). The lesions observed in the thymus were primarily a reduction of the cortical area and in some cases a focal depletion of thymocytes in the cortex. Cyst formation around Hassall bodies was infrequent, and in a few cases the cortical area of thymus assumed a nodular appearance. Pyknotic thymocytes were also present.

TABLE 2  
INCIDENCE OF DISCERNIBLE LESIONS IN THE LIVER AND THYMUS  
OF MALE MICE EXPOSED TO TCDD\*

| Exposure<br>Level<br>( $\mu\text{g/kg/week}$ ) | Organ   | Duration of Exposure |         |         |
|------------------------------------------------|---------|----------------------|---------|---------|
|                                                |         | 2 Weeks              | 4 Weeks | 8 Weeks |
| 0 (Control)                                    | Liver†  | 2/4                  | 1/3     | 3/4     |
|                                                | Thymus  | 1/4                  | 0/3     | 0/4     |
| 0.01                                           | Liver†  | 0/4                  | 1/4     | 4/4     |
|                                                | Thymus  | 1/4                  | 0/4     | 2/4     |
| 0.1                                            | Liver‡  | 1/3                  | 2/4     | 4/4     |
|                                                | Thymus  | 0/4                  | 1/4     | 2/4     |
| 1                                              | Liver‡  | 4/4                  | 4/4     | 4/4     |
|                                                | Thymus§ | 0/4                  | 3/4     | 1/4     |
| 10                                             | Liver‡  | 4/4                  | 4/4     | 4/4     |
|                                                | Thymus§ | 1/4                  | 4/4     | 0/4     |

\*Sections examined microscopically in a blind random fashion. Only data on liver and thymus are summarized here since kidney showed no alterations in any group. The numbers indicate the number of animals showing abnormalities/total number of animals in that group.

†The hepatic lesions in control and those receiving 0.01  $\mu\text{g/kg/week}$  group involved vacuolation or slight degeneration of hepatic cells.

‡The organs showed extensive perilobular diffuse degeneration and/or necrosis.

§Marked cortical atrophy of thymus with or without the depletion of thymocytes was noticed in these groups. Other groups did not show any definite lesions.

TCDD caused a stimulation of lymphocyte transformation when no mitogens were present in the culture system (FIGURE 1). After 4 weeks of TCDD administration, there was a significant increase in the thymidine uptake of splenic cultures at all TCDD dose levels as compared to controls. This trend appeared to be reduced after 8 weeks of exposure.

The extent of phyto mitogen (PHA or PWM)-induced lymphocyte blast formation was reduced by TCDD exposure (FIGURE 2). The decrease in the stimulation indices (DPM in the presence of a phyto mitogen divided by the DPM in an identical culture but without the mitogen) in TCDD groups is partly because of the increased thymidine uptake in unstimulated cultures. A reduction seen in stimulation indices after 2 weeks of TCDD exposure became marked after 4 weeks and seemed to

disappear at 8-week period. The decrease was observed both in PHA- and PWM-induced-stimulation indices, and a significant reduction (in paired comparisons) was observed in one or more dose levels at different time intervals despite the large variation inherent in this type of assay system.<sup>10</sup>

The serum immunoglobulins in mice at different periods of TCDD exposures are shown in TABLE 3. The treatment with 0.01 or 0.1  $\mu\text{g}$  TCDD/kg/week appeared to increase the immunoglobulin, while the higher doses (i.e. 1 and 10  $\mu\text{g}$ /kg/week) of TCDD decreased these proteins.

When mice were treated orally with a single dose (10  $\mu\text{g}$ /kg) of TCDD and sacrificed at 2, 4, and 8 weeks after the treatment, a significant increase in spontaneous lymphocyte transformation was seen only at 2 weeks after the challenge. The details of these results have been presented elsewhere.<sup>11</sup> The effects on

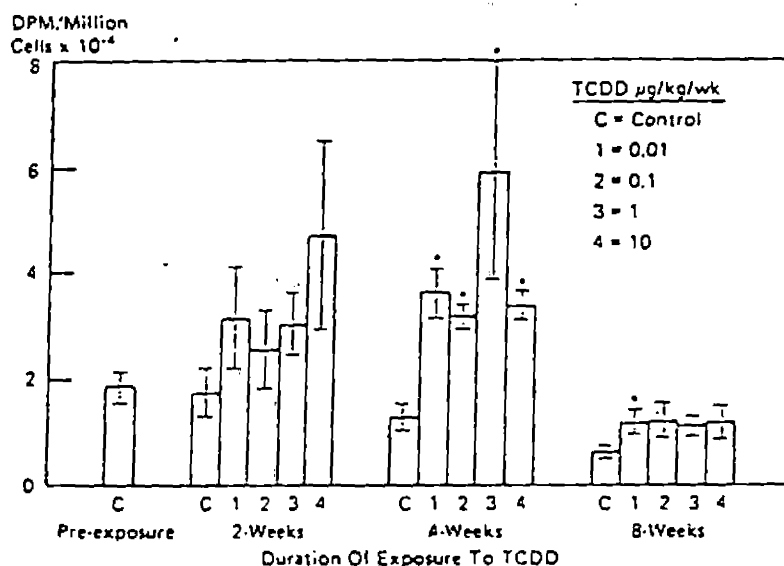


Figure 1. Uptake of <sup>3</sup>H-thymidine by unstimulated mouse splenic cultures at different periods after exposure to TCDD. Although a decrease with time is seen in control cultures, all TCDD-exposed groups showed an increase over respective control groups. \*Indicates a significant deviation from control ( $p < 0.05$ ).

lymphocyte transformation, apparent at 2 weeks post-administration persisted at the 4-week interval after treatment but were not significant, and no differences between control and treated animals were observed in cultures 8 weeks after doing. A similar trend was observed in the respective stimulation indices. The thymic changes and the increase in liver weights also followed a similar trend. The histologic effects in liver, however, persisted, although the degenerative lesions observed microscopically were less severe after 8 weeks.

When TCDD was directly added to the media in splenic cultures, the lymphocytes did not show any increased blastogenic activity. In fact, a dose-related decrease in <sup>3</sup>H-thymidine incorporation by lymphocytes was observed (FIGURE 3) with increasing TCDD concentrations. FIGURE 4 presents data on a similar system when the cells were

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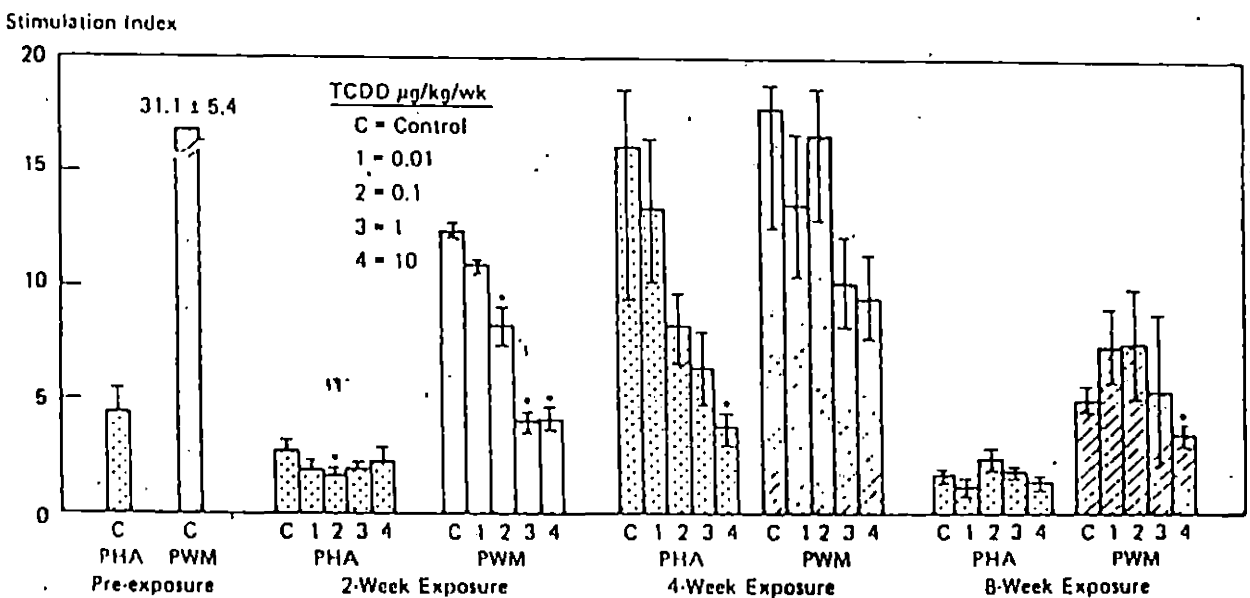


FIGURE 2. Stimulation indices by phytoimitogens, phytohemagglutinin (PHA) and pokeweed mitogen (PWM) in mouse splenic cultures in relation to TCDD exposure. The cultures are similar to those in FIGURE 1. \*Indicates significant difference in group when compared to respective controls ( $p < 0.05$ ). Stimulation index = DPM from a culture in the presence of either PHA or PWM divided by the DPM in a similar culture without a mitogen.

TABLE 3  
SERUM-IMMUNOGLOBULINS IN MICE EXPOSED TO DIFFERENT LEVELS OF TCDD

| TCDD Exposure ( $\mu\text{g/kg/wk}$ ) | Precapture      | 2 Weeks         | Serum Ig at* 4 Weeks | 8 Weeks              |
|---------------------------------------|-----------------|-----------------|----------------------|----------------------|
| 0                                     | $0.49 \pm 0.06$ | $0.94 \pm 0.25$ | $1.14 \pm 0.06$      | $0.91 \pm 0.12$      |
| 0.01                                  | —               | $1.06 \pm 0.11$ | $1.48 \pm 0.16$      | $1.95 \pm 0.99$      |
| 0.1                                   | —               | $1.04 \pm 0.15$ | $1.67 \pm 0.39^{**}$ | $2.58 \pm 0.47^{**}$ |
| 1                                     | —               | $1.17 \pm 0.27$ | $1.43 \pm 0.18$      | $0.85 \pm 0.11$      |
| 10                                    | —               | $0.87 \pm 0.13$ | $0.79 \pm 0.08^{**}$ | $0.72 \pm 0.18$      |

\*The values expressed are ratios of serum Ig in relation to a pooled normal mouse serum sample. Mean  $\pm$  S.E. of 4 observations per group.

\*\*Indicate significant difference from the respective control at  $p < .05$ .

cultured in the presence of mitogenic agents. No effect of TCDD on stimulation indices suggest that the observation in FIGURE 3 was possibly due to the direct cytotoxic effect of TCDD on lymphocytes.

#### DISCUSSION

Our experiments with TCDD in mice confirm the early findings that high dose levels of TCDD are immunosuppressive. It has been shown to be an immunosuppressant in guinea pigs, and rats<sup>1</sup> and developing rats and mice after maternal and

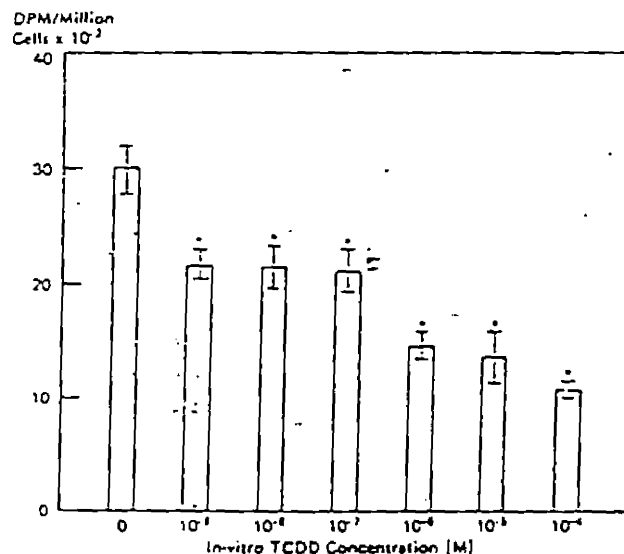


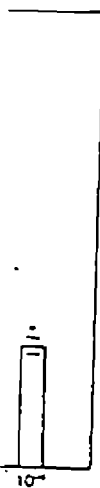
FIGURE 3. Effect of TCDD added directly to the incubation medium on lymphocyte transformation in mouse splenic lymphocyte cultures. Cells isolated from normal mice were incubated in culture media containing appropriate concentrations of TCDD. \*Indicates significant difference from the control cultures at  $p < 0.05$ .

## Levels of TCDD

| 8 Weeks |                      |
|---------|----------------------|
|         | $0.91 \pm 0.12$      |
|         | $1.95 \pm 0.99$      |
| --      | $2.58 \pm 0.47^{**}$ |
|         | $0.85 \pm 0.11$      |
| --      | $0.72 \pm 0.18$      |

cd normal mouse serum

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postnatal treatment.<sup>4</sup> The immunosuppression in our experiments was evident by decreased stimulation indices of splenic lymphocytes to the mitogenic agents, PHA and PWM.

The results presented in this report, however, differ from the earlier ones in several respects. The immunosuppressive effect of TCDD in mice was not necessarily dose-related in the range of concentrations that we have used. Usually the pathologic lesions were apparent, particularly in the liver, at levels of TCDD exposure lower than that were shown to be immunosuppressive. Generally the immune suppression was seen in mice at doses of 1  $\mu\text{g/kg/wk}$  or more, when exposure was continued for several weeks. At lower exposure levels, the immunosuppressive effect was not apparent while liver changes were observed. The changes in thymus, described in earlier reports<sup>5</sup> were

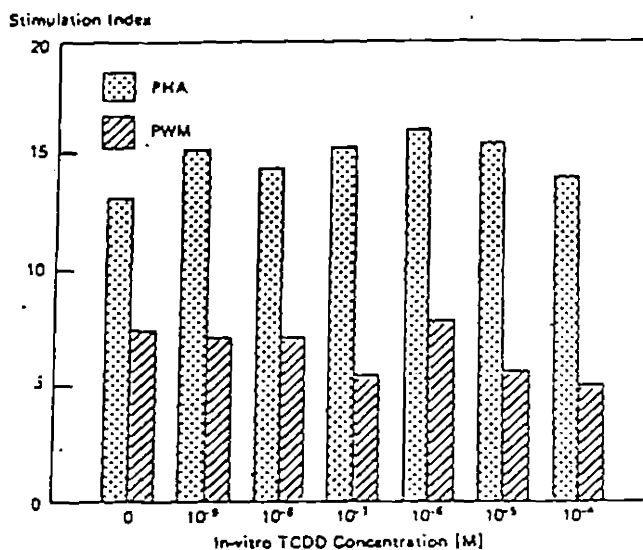


FIGURE 4. Stimulation indices in splenic lymphocyte cultures incubated in the presence of PHA or PWM and different concentrations of TCDD. The cultures are similar to those indicated in FIGURE 3. Stimulation index = DPM from a culture in the presence of either PHA or PWM divided by the DPM in an identical culture without mitogens. Addition of TCDD did not have any effect on stimulation indices by either PHA or PWM.

observed in our studies, but these were not always dose-related. One unique feature of such thymic lesions was the absence of any changes in the two highest dose levels of TCDD exposure at the end of 8 weeks in mice. The only appreciable atrophy of thymic cortex and depletion of thymocytes were seen in these treatment levels at the end of 4 weeks. In mice, even the decrease in thymus weights that was observed after 2 and 4 weeks of TCDD exposure, was not seen after 8-week treatment. Although not conclusive, this finding suggests that there may be a recovery of thymic tissue even if the exposure to TCDD continued at high levels. It may be interpreted as a sort of adaptation in this organ to the presence of TCDD. The same was not true, however, for liver lesions, which were progressive with time and also with the level of TCDD exposure.

An important observation of this study is an increase in the spontaneous blast

formation in splenic lymphocytes without any mitogenic inducer. This increase was consistent at all time periods and particularly obvious in mice receiving low dose levels of TCDD. The exact implication of this finding is not clear, but it suggests a stimulation in the functional activity of lymphocytes. This can be compared to an increase when the animals are challenged with an antigen.<sup>12</sup> In our additional studies, such increase was seen in control groups that were injected with an antigen mixture (tetanus toxoid and Freund's adjuvant; data not given here). One possible explanation of this stimulation in spontaneous lymphocyte transformation may be that the administration of TCDD produces an antigenic response, either by combining with a body protein, or by causing tissue damage leading to the release of altered proteins that might be antigenic. Poland and Glover<sup>13</sup> have reported a specific and high affinity binding of TCDD with hepatic cytosol.

The decrease in the stimulation indices to mitogens by TCDD exposure may be the result of immune tolerance produced by the possible antigenic effect of this chemical. Both antigens and antibodies are known to produce immune tolerance or immune unresponsiveness.<sup>14</sup> In our studies, an immune unresponsiveness (or immune suppression) was seen only at relatively high exposures to TCDD, which may suggest that a saturable amount of either antigen or antibody may be present at these treatment levels.

The hypothesis of an autoimmune response is also supported by the finding that in mice TCDD produced an increased immunoglobulin synthesis at the low levels of exposure. A significant increase in total Ig appeared at 4 and 8 weeks after 0.1  $\mu\text{g/kg/week}$  of TCDD treatment. At high treatment levels of TCDD, the decrease in immunoglobulin levels may be related to the damage of the organs or cells that are sites of globulin production (e.g. lymphoid tissue). 11/17

Vos and Moore<sup>4</sup> have reported that PHA responsiveness on splenic cells was decreased in one-month-old mice but not in 4-month-old animals. In our studies using 4-5 month old mice (data not shown) both PHA and PWM responsiveness were significantly decreased 2 weeks after a single dose of 10  $\mu\text{g/kg}$  TCDD, although this decrease was not as remarkable as in growing animals. In these animals as well, the spontaneous increase in DNA synthesis was seen in splenic cultures. One of the reasons for the relative insensitivity of adult animal lymphocytes may be that the lymphocyte response to mitogens as well as spontaneous blast formation in culture is considerably decreased with age and the influence of the chemical treatment may not be remarkable. Adult animals may also bind TCDD more efficiently in selected organs (i.e. liver) than in the young ones, thus there may be less amount of TCDD available in the adults in other tissues.

#### SUMMARY

Male CD-1 mice were orally treated with 0.01, 0.1, 1 and 10  $\mu\text{g}$  TCDD/kg body wt./week for up to 8 weeks. Randomly selected animals were sacrificed at 2, 4, and 8 weeks of exposure. An additional group was given orally 10  $\mu\text{g}$  TCDD/kg and the animals similarly sacrificed. Splenic lymphocytes from these animals were cultured *in vitro* with or without the presence of phyto mitogens, phytohemagglutinin, and pokeweed mitogen. The incorporation of  $^3\text{H}$ -thymidine was measured as an indication of relative blast formation. Exposure of animals to TCDD, even at the lowest level (0.01  $\mu\text{g/kg/wk}$  for 2 weeks) caused a marked increase in the thymidine uptake by cultured lymphocytes. The blastogenic response of mitogens was reduced at high levels of TCDD exposure, indicating an immunosuppressive effect. Following a single treatment with 10  $\mu\text{g}$  TCDD/kg, the increase in the blast formation was noted at 2

ducer. This increase was receiving low dose levels clear, but it suggests a can be compared to an our additional studies, with an antigen mixture. One possible explanation may be that the her by combining with a release of altered proteins specific and high affinity

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ed by the finding that in sis at the low levels of and 8 weeks after 0.1 TCDD, the decrease in organs or cells that are

ss on splenic cells was als. In our studies using M responsiveness were g TCDD, although this ese animals as well, the e cultures. One of the eocytes may be that the formation in culture is ical treatment may not efficiently in selected less amount of TCDD

10 µg TCDD/kg body sacrificed at 2, 4, and 8 µg TCDD/kg and the imals were cultured in tohemagglutinin, and ssured as an indication on at the lowest level - thymidine uptake by reduced at high levels . Following a single tation was noted at 2

weeks, the effect reduced at 4 weeks, and no difference noticed in treated vs. control cultures 8 weeks after the treatment. Small doses of TCDD stimulate the splenic lymphocyte transformation and this effect, although somewhat dose-related, can be reversed in a relatively short period of time.

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