

Increased Contraction Frequency in Rat Uterine Strips Treated *In Vitro* with *o,p'*-DDT

Daland R. Juberg and Rita Loch-Carusio

The Toxicology Program of the Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan 48109-2029, USA

Elevated levels of DDT and other organochlorine pesticides have been associated with spontaneous abortion and preterm birth in several species, including humans (DeLong et al. 1973; Hart et al. 1971; O'Leary et al. 1970; Saxena et al. 1980, 1981; Wassermann et al. 1982). Despite the prevalence of organochlorine pesticides in our environment, a mechanistic basis for this association has not been explored. Furthermore, while DDT has been associated with inhibition of calcium ATPases (Ghiasuddin and Matsumura 1981; Treinen and Kulkarni 1986), altered gap junctional communication (Madhukar et al. 1983; Davidson et al. 1985), and electrophysiological changes (Schefczik and Buff 1984; Beeman 1982), all of which could affect the excitation-contraction process characteristic of smooth muscle, direct effects of DDT on uterine smooth muscle have not been reported. This study was initiated to assess the direct effects of *o,p'*-DDT (an estrogenic isomer present in the technical grade preparation) on pregnant rat uterine tissue.

MATERIALS AND METHODS

o,p'-DDT (99% pure; Crescent Chemical Co., Hauppauge, NY) was dissolved in absolute ethanol to make a stock solution of 20 mg/ml. Pregnant Sprague-Dawley rats were killed on day 10 of gestation by ether anesthesia followed by exsanguination. The uteri were removed and placed in a physiological saline solution (PSS; 130 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4 , 1.1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 14.9 mM NaHCO_3 , 5.5 mM dextrose, 0.027 mM EDTA, 1.6 mM CaCl_2). Uterine strips (2 mm x 10 mm) were cut along the longitudinal axis and placed in PSS containing either 100 μM *o,p'*-DDT or 0.17% (v/v) ethanol (solvent control) overnight (12-23 hr) at 4°C. The following day the strips were mounted in 50-ml muscle bath chambers which contained PSS, and bubbled with 5% $\text{CO}_2/95\%$ O_2 at 37°C. Strips were allowed to equilibrate to the experimental conditions for 1 hr in the continued presence of 100 μM DDT or 0.17% ethanol (solvent control). The chambers were rinsed twice and an initial passive tension of 0.5 g was placed on each strip. After another hour of equilibration, isometric contraction activity was

Send reprint requests to R. Loch-Carusio at the above address.

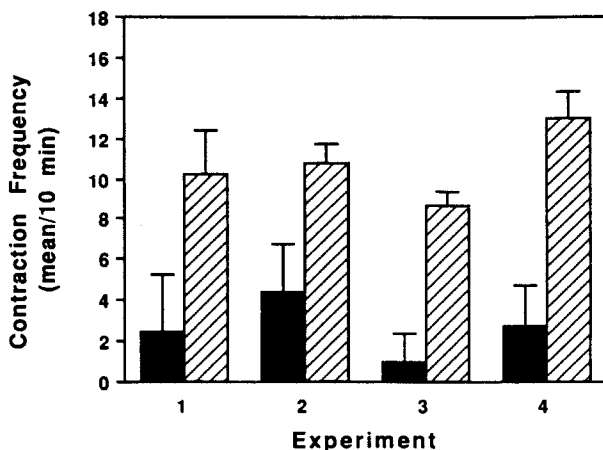


Figure 1. Isometric contraction frequency is significantly increased in rat uterine strips treated *in vitro* with 100 μM o,p'-DDT (hatched bars) compared to controls (solid bars), $p < 0.001$. Contractions were monitored for 60 min. There were two uterine strips per treatment for each of four experiments.

monitored and recorded with force transducers attached to a Grass polygraph. The chambers were rinsed at 30-min intervals to maintain pH and buffer capacity. Data were collected for a 60-min period following the 2 hr equilibration, during which contraction frequencies were quantified for control and treated strips. Data were analyzed using a paired t-test design.

RESULTS AND DISCUSSION

Significant increases in isometric contraction frequency were observed over the 60-min test period in rat uterine strips treated *in vitro* with 100 μM o,p'-DDT (Fig. 1). In each of four experiments, two uterine strips were included in each treatment group (Fig. 1). Increased contraction frequency was observed in DDT-treated strips throughout the experimental period, including the 60-min equilibration period with passive tension, as shown in the example provided in Figure 2.

Figure 3 is a polygraph tracing showing the increased activity of DDT-treated strips. While control strips often displayed periods of inactivity, this was not the case with DDT-treated strips, nor was any decrease in contraction frequency observed throughout the experimental period.

Assessment of uterine activity may be relevant for investigations of preterm birth. Several reports indicate that the frequency of uterine contractions is significantly greater in women who deliver prematurely compared to women who deliver at term (Bell 1983; Katz et al. 1986). Additional studies suggest that a contraction pattern characterized by low-amplitude, high-frequency contractions

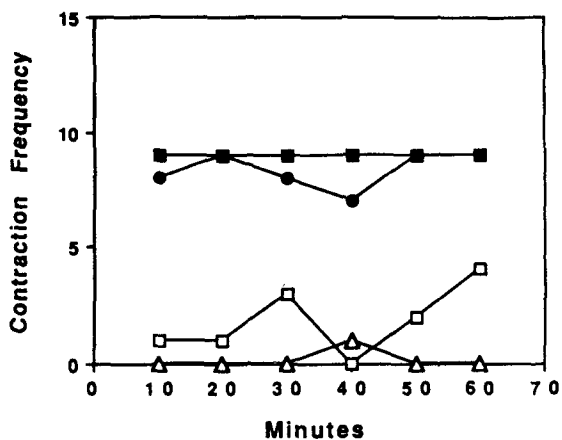


Figure 2. Rat uterine strips treated *in vitro* with 100 μM o,p'-DDT (solid symbols) show increased contractile activity throughout the 1 hr experimental period compared to controls (open symbols). Each point represents the cumulative number of contractions per preceding 10 min period. From experiment 3 of Figure 1.

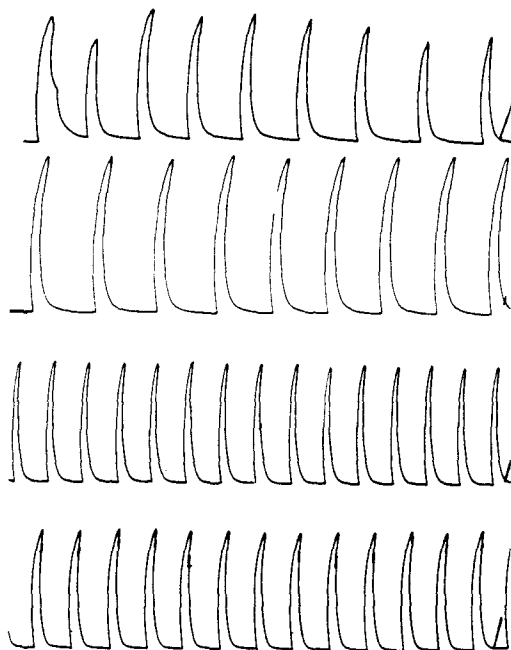


Figure 3. Polygraph tracings of rat uterine contractile activity showing increased contraction frequency in DDT-treated strips (bottom two tracings) and controls (top two tracings).

is exhibited significantly more often in women destined to develop preterm labor than in women who deliver at term (Newman et al. 1987). In fact, subsequent to this study, we have observed contractile activity among DDT-treated rat uterine strips which resembles a low-amplitude, high-frequency pattern (unpublished observation).

Using a modified test procedure, on-going studies in our laboratory have confirmed the increased contractile activity reported here. We currently are characterizing dose-response and gestational age effects, and are investigating a mechanistic basis for the increased contraction frequency observed in DDT-treated rat uterine strips. To our knowledge, the direct effects of DDT (or any other organochlorine pesticide) on uterine contractility have not been reported. Consequently, these data may help to establish a basis for the association between organochlorine pesticide blood levels and preterm birth.

Acknowledgments. We thank Dr. R. Clinton Webb for technical assistance and laboratory use and Dr. Craig Harris for providing animal tissue. This research was supported by predoctoral training grants to DRJ (NIH GM07767 and NIH HD07048), a research grant to RLC (NIH ES04424), and the Laboratory Animal Core of the P30 Center for the Study of Reproduction (NIH HD18258).

REFERENCES

- Beeman RW (1982) Recent advances in mode of action of insecticides. *Annu Rev Entomol* 27:253-281
- Bell R (1983) The prediction of preterm labor by recording spontaneous antenatal uterine activity. *Br J Obstet Gynecol* 90:884-887
- Davidson JS, Baumgarten I, Harley EH (1985) Use of a new citrulline incorporation assay to investigate inhibition of intercellular communication by 1,1,1-trichloro-2,2-bis (p-chlorophenyl)-ethane in human fibroblasts. *Cancer Res* 45: 515-519
- DeLong RL, Gilmartin WG, Simpson JG (1973) Premature births in California sea lions: Associations with high organochlorine pollutant residue levels. *Science* 181:1168-1170
- Ghiasuddin SM, Matsumura F (1981) DDT inhibition of Ca-Mg ATPase from peripheral nerves and muscle of lobster *Homarus Americanus*. *Biochem Biophys Res Commun* 103:31-37
- Hart MM, Adamson RH, Fabro S (1971) Prematurity and intrauterine growth retardation induced by DDT in the rabbit. *Arch int Pharmacodyn* 192:286-290
- Katz M, Newman RB, Gill PJ (1986) Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery. *Am J Obstet Gynecol* 154:44-47
- Madhukar BV, Yoneyama M, Matsumura F, Trosko JE, Tsushimoto G (1983) Alteration of calcium transport by tumor promoters, 12-o-tetradecanoylphorbol-13-acetate and p,p'-dichlorodiphenyltrichloroethane, in the Chinese hamster V79 fibroblast cell line. *Cancer Lett* 18:251-259

- Newman RB, Gill PJ, Campion S, Katz M (1987) Antepartum ambulatory tocodynamometry: The significance of low-amplitude, high-frequency contractions. *Obstet Gynecol* 70:701-705
- O'Leary JA, Davies JE, Edmundson WF, Feldman M (1970) Correlation of prematurity and DDE levels in fetal whole blood. *Amer J Obstet Gynecol* 106:939
- Saxena MC, Siddiqui MKJ, Bhargava AK, Seth TD, Krishnamurti CR, Kutty D (1980) Role of chlorinated hydrocarbon pesticides in abortions and premature labor. *Toxicology* 17:323-331
- Saxena MC, Siddiqui MKJ, Seth TD, Krishnamurti CR, Bhargava AK, Kutty D (1981) Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature, or full-term delivery. *J Anal Toxicol* 5:6-9
- Schefczik K, Buff K (1984) The insecticide DDT decreases membrane potential and cell input resistance of cultured human liver cells. *Biochem Biophys Acta* 776:337-339
- Treinen KA, Kulkarni AP (1986) Human placental Ca^{2+} -ATPase: *In vitro* inhibition by DDT homologs. *Toxicol Lett* 30:223-229
- Wassermann M, Ron M, Bercovici B, Wassermann D, Cucos S, Pines A (1982) Premature delivery and organochlorine compounds: Polychlorinated biphenyls and some organochlorine insecticides. *Environ Res* 28:106-112

Received October 8, 1990; accepted October 29, 1990.