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LETTER

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## REPROTOX AGENT REQUEST: 2514

DDT (dichlorodiphenyltrichlorethane) is an organochlorine insecticide. Technical DDT is a mixture of p,p'-DDT and related compounds, including DDE and DDD. One of the more important of the DDT isomers is o,p'-DDT. These agents have prominent estrogenic effects that have been well characterized in a number of assay systems (1-6). The estrogenicity of DDT and DDE has led to the supposition that they may adversely affect reproductive outcome, either by causing birth defects, by increasing pregnancy complications, or by affecting fertility.

Chick embryos exposed to DDT were reported in one study to have an increased incidence of CNS and circulatory anomalies (7) and in another to demonstrate abnormal development of the gonads (8). DDT has been shown to cross the placenta in rats (9) but teratogenicity studies in this species have been negative (10,11). No increase in malformations were reported in the offspring of treated mice (12), although abnormal gonadal function was seen in another study in the F1 generation (13). DDT administered to pregnant rabbits has been shown to cross the placenta and to cause growth retardation of the fetus and preterm delivery but no increase in birth defects in the offspring (14,15). Early in rabbit pregnancy, administration of DDT to the doe results in appearance of the compound in uterine fluid and access to the blastocyst (16,17) and growth retardation may result from such exposure (18). Decreased viability of offspring from DDT-treated pregnancies have been noted in rats (10). An inhibition of implantation was found in mice given high doses of DDT (19); by contrast, o,p'-DDT and DDE supported implantation and continued pregnancy in hypophysectomized rats (1,30). This effect was attributed to the estrogenicity of these compounds. Other studies have not found adverse effects of DDT exposure on pregnancy outcome parameters such as litter size and survival in rats (20,21), mice (22,23), and dogs (24).

DDT and DDE are known to cross the human placenta at term (25,31,32) and these compounds can be found in the tissues of mother and newborn. A few reports have evaluated a possible role for organochlorine pesticides, including DDT and DDE, in adverse human

pregnancy outcome. In a study comparing the levels of DDT and its congeners in the blood and placentae from women with preterm birth or miscarriage versus women delivering normally at term, Saxena and coworkers reported significantly higher levels associated with the adverse outcomes (26,34). Similar findings were reported by the same investigators using milk DDT levels as a measure of pesticide body burden (27). These authors also noted higher concentrations of DDT in maternal serum, cord blood, and placentae from stillborn pregnancies than from matched controls (36). A different group found higher proportions of o,p'-DDT (compared to total DDT) in women who had spontaneous abortions than in women with normal pregnancies (33). By contrast, another study reported that serum levels of DDT and its metabolites were not different between 120 women hospitalized for miscarriage and 120 women delivering at full term (28). In a small sample of women with premature rupture of the membranes, no difference in levels of DDT or related compounds in maternal or cord blood were found compared to controls (29), but DDT concentrations were higher in women delivering preterm than in women delivering at term (34). The role, if any, of DDT, DDE, and related compounds in human pregnancy complications is considered unclear.

A number of studies have documented that compounds of the DDT group, and particularly DDE, enter human breast milk (37-51). The concentration of these chemicals is highest in areas in which DDT was extensively used. Some studies also have found an correlation between milk concentrations and maternal age. In one study on colostrum, total DDT concentrations were calculated to be high enough in 70% of samples to produce neonatal exposures above the Acceptable Daily Intake (ADI) values established by the World Health Organization (51). In addition, the DDT body burden of young children appears to be associated with the quantity of mothers' milk consumed (52). Although this has produced concern, it has been difficult to document adverse effects of this neonatal exposure. One group used neurodevelopmental tests to assess children exposed either transplacentally, or by milk, and found no persistent decrements when compared to controls (53,54), although DDE exposure was said to be transiently associated with hyporeflexia (55). There was no association with birth weight or head circumference in this study (55). It has been noted that women with high concentrations of DDE in their milk nurse their children for much shorter periods of time than do women with low concentrations and it has been proposed that DDE inhibits lactation (56).

The potential lactation effects of DDT and related compounds have been evaluated in animals models. The observation that DDE may inhibit lactation was investigated by treating rats with 10 mg/kg/day (5 days/week) from before pregnancy, through gestation, and throughout the lactation period. This dose schedule resulted in milk DDE concentrations two orders of magnitude higher than those found in women; however, there were no adverse effects on milk composition, pup survival, or weight gain (57). In a mouse study, neonates were fed

DDT, DDE, and DDD in amounts up to 100 times the estimated human neonatal daily consumption. Proliferation of smooth endoplasmic reticulum in the liver cells was seen (58).

Although the estrogenic activity of DDT and its congeners might theoretically affect fertility of men or women exposed to this agent, there are few data on this issue. Chlorinated hydrocarbons were found in seminal fluid and cervical mucus of infertility patients, prompting the authors to suggest a role for pollutants in reproductive problems (59). DDE but not DDT has been found in testicular biopsy material (60), but in a controlled evaluation of semen samples, the concentration of DDE did not appear to be related to semen quality or a history of infertility (61).

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# REPRODUCTIVE TOXICOLOGY

## a medical letter

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on Environmental Hazards to Reproduction

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### INSECTICIDES

Modern insecticide development began at the time of World War II. One of the first agents to be synthesized was 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, known more simply as DDT (dichlorodiphenyltri chloroethane). Production of DDT reached peak annual levels of a quarter of a million tons in 1964. Appreciation of the toxicity and persistence of this compound led to its ban in the United States in 1972 (it is still in use in many tropical countries). Hundreds of other insecticidal chemicals remain in widespread use, however, and human contact with these agents is commonplace. The heaviest exposure occurs in agricultural and industrial settings; however, the use of insecticides to control household pests results in the exposure of many individuals in the home. If these agents are reproductive toxins, their prevalence in the environment would constitute an important public health problem.

**CHEMISTRY AND MECHANISMS OF ACTION** — Insecticides are divided into groups based on similarities of structure. The earliest of the compounds to be synthesized were organochlorines. These are hydrocarbons (often aromatic) with chlorine substitutions and include DDT, aldrin, dieldrin, endrin, telodrin, chlordane, heptachlor, endosulfan, chlordecone (Kepone), mirex, toxaphene, paradichlorobenzene, and lindane (discussed in **Reproductive Toxicology** 2:11, 1983). These compounds are virtually insoluble in water but are highly lipid soluble. In biological systems they can accumulate in fats. Vertebrates are capable of extensive metabolism of these chemicals; however, the metabolites are often as toxic as the parent compound. Complete degradation of the organochlorines is unusual and these insecticides persist in the environment for many years. A mechanism for the insecticidal properties of chlorinated hydrocarbons has not been established; however, it does not appear to involve cholinesterase inhibition (the mechanism of action for most other insecticides). For example, the most thoroughly investigated of these agents, DDT, appears to alter the electrophysiological properties of neuronal cell membranes.

The organophosphorous insecticides are derivatives of phosphoric or phosphonic acid. In the subset of these agents known as the thiophosphates, the oxygen of the phosphoric acid moiety is replaced by sulfur. The organophosphates readily hydrolyze in water which explains their lack of persistence in the environment. Agents in this group include dichlorvos (No Pest Strip), chlorfenvinphos, tetrachlorvinphos, crotoxyphos, mevinphos, phosphamidon, monochrotophos, dichrotophos, parathion, fenitrothion, bromophos, iodofenphos, fenthion, cyanophos, abate, pirimiphos, diazinon, chlorpyrifos, azinphos, methidathion, dimethoate, amiphos, formothion, kitazin, vamidothion, oxydemeton, crufomate (rueleene), and trichlorfon. In spite of the diversity in structure of these compounds, they all appear to work by phosphorylating acetylcholinesterase, thus preventing degradation of acetylcholine. In insects acetylcholine is an important central

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neurotransmitter and acetylcholinesterase inhibition results in paralysis of the animal. Mammals are able to rapidly metabolize the organophosphate insecticides; however, these agents are capable of inhibiting cholinesterases in all species.

The carbamate insecticides are derivatives of carbamic acid. Although they are only slightly soluble in water, they are readily oxidized in the presence of light, heat, and air and so do not persist in the environment. Carbamates include carbaryl (Sevin), carbofuran, propoxur (Baygon), landrin, aminocarb, methomyl, and dimetilan. These compounds are readily metabolized by mammals. Insecticidal action is mediated by carbamoylation of acetylcholinesterase with resultant enzyme inhibition.

There are other insecticides in use which cannot be classed in the above manner. Nicotine binds to post-synaptic membranes in the insect central nervous system and is used as a solution or as a soap in insect control. Rotenone is extracted from the root of a plant. It blocks mitochondrial respiration in nerve and muscle. Pyrethroids are complex chemicals derived from flowers. Pyrethrum, a commonly encountered agent in this group, is a mixture of at least six different compounds. Pyrethroids appear to induce the production of an as-yet unidentified neurotoxin in susceptible insects (Moutschen-Damen and Degraeve, in Kirsch-Volders [ed]: *Mutagenicity, Carcinogenicity, and Teratogenicity of Industrial Pollutants*, NY, Plenum Press, 1984, pp 127-203).

Investigation into the potential human reproductive impact of insecticides is made difficult by the inability to quantitate the degree of exposure encountered in real-life situations. Commercial products often contain mixtures of several insecticides and by-products of insecticide synthesis. In addition, solutions of these agents frequently contain organic solvents which may have independent reproductive effects (see *Reproductive Toxicology* 2:17, 1983). Most of the information available is derived from studies of purified, single agents applied to selected *in vitro* or animal systems.

**MUTAGENICITY AND CLASTOGENICITY** — The ability of some insecticides to alter the heritable characteristics of organisms or to disrupt chromosomes has been demonstrated (Moutschen-Dahmen and Degraeve, *op cit*). Whether the ability to alter genetic material results in reproductive toxicity in humans is unknown. About 20% of organophosphorus insecticides are mutagenic in bacterial tests (Hanna and Dyer, *Mutat Res* 28:405, 1975). Of these, mutagenicity has been most thoroughly studied with dichlorvos (Bridges et al, *Mutat Res* 19:295, 1973). Negative bacterial tests have been noted for most organochlorines, the carbamates, pyrethroids, and rotenone. Most insecticides tested are negative or weakly mutagenic in eukaryotic test systems. DDT, for example, causes chromosomal breakage in marrow cells of mice treated with 200-400 mg/kg but not with 100-150 mg/kg (Johnson and Jalal, *J Hered* 64:7, 1973). Aldrin and dieldrin, closely-related organochlorines, show definite mutagenic effects in some but not all assays (reviewed by Ashwood-Smith, *Mutat Res* 86:137, 1981). Mouse dominant lethal data suggest that organochlorine insecticides are not important genetic toxins (Epstein et al, *Toxicol Appl Pharmacol* 23:288, 1972). One study of three organochlorine insecticides, DDT, chlordane, and lindane, simultaneously examined three aspects of abnormal cell replication and organization in culture: cytotoxicity, mutagenesis, and cell-cell interaction. All three compounds were found to be cytotoxic, none were mutagenic, and DDT and lindane were found to disrupt cell-cell communication (Tsushimoto et al, *Arch Environ Contam Toxicol* 12:721, 1983). Many organophosphorus insecticides have been shown to be mutagenic and/or clastogenic in a number of eukaryotic systems but the results of different studies conflict and positive findings are seen only at high doses (Moutschen-Dahmen and Degraeve, *op cit*).

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There are only a few reports on possible genotoxic effects in human populations exposed to insecticides. Workers in direct contact with DDT were compared with people working in the same plant but without such contact. There was no difference in the number of chromosomal aberrations in cultured lymphocytes between the groups. Five of the "non-exposed" controls in one plant were found to have elevated DDT plasma levels, however. When these five workers were included in the "exposed" group, a significant elevation in chromosomal abnormalities was associated with DDT exposure (Rabello et al, *Mutat Res* 28:449, 1975). A similar study performed in dieldrin workers showed no increase in aberrant chromosomes associated with insecticide exposure (Dean et al, *Food Cosmet Toxicol* 13:317, 1975). Organophosphate workers were found to have an increased incidence of chromosomal abnormalities when compared to controls; however, the difference between the groups was not statistically significant (Király et al, *Arch Environ Contam Toxicol* 8:309, 1979). Finally, lymphocyte karyotypes were obtained from pesticide applicators during the peak season and the off season. Pesticides included a number from different chemical classes. There was an increase in the incidence of chromatid breaks when peak season karyotypes were compared to those from the off season. Chromatid gaps, however, decreased in the same comparison (Yoder et al, *Mutat Res* 21:335, 1973).

**FERTILITY EFFECTS** — Many animal reproduction studies using high doses of insecticides showed decreased pregnancy rates attributable to illness of the adult animal; however, it appears that some organochlorines may have independent fertility effects. DDT, DDT analogues, lindane, and aldrin, for example, are estrogenic and may disturb female sexual function in some species if given in high doses (Hayes, *Pesticides Studied In Man*, Baltimore, Williams & Wilkins, 1982). Chlordecone, another organochlorine, exerts effects on the genitalia of female neonatal mice similar to those of estradiol (Eroschenko and Mousa, *Toxicol Appl Pharmacol* 49:151, 1979) and can cause constant estrus in adult mice (reviewed in Hayes, *op cit*). There is no information, however, showing adverse fertility effects in women exposed to organochlorine insecticides. Mirex, an organochlorine structurally similar to chlordecone, causes a decrease in sperm concentration in male kestrels (Bird et al, *Arch Environ Contam Toxicol* 12:633, 1983). Very high doses of DDT can also cause a decrease in avian testicular size, presumably due to the compound's estrogenic properties (Hayes, *op cit*). Carbaryl, a carbamate insecticide, has been reported in the Russian literature to be a testicular toxin in animals (reviewed by Whorton et al, *J Toxicol Environ* 5:929, 1979); however, mice exposed to carbaryl at doses up to 34 mg/kg/day for 5 days do not show decreased testicular weight or a decreased assimilation of testosterone by the prostate (Thomas et al, *Toxicol Appl Pharmacol* 28:142, 1974). A study of male carbaryl workers showed no association between insecticide exposure and semen abnormalities or levels of follicle stimulating hormone, luteinizing hormone, or testosterone (Whorton et al, *op cit*). A comparison of these workers to newly hired men showed more abnormally shaped spermatozoa in men with carbaryl exposure (Wyrobek et al, *Environ Health Perspect* 40:255, 1981).

**EMBRYOTOXICITY AND TERATOGENICITY** — Many insecticides and/or their metabolites gain access to the conceptus after dosing of the mother. Single doses of carbamate insecticides given to pregnant rats result in decreased fetal acetylcholinesterase levels in blood, brain, and liver within 5 minutes which persist for as long as 24 hours (Cambon et al, *Toxicol Appl Pharmacol* 49:203, 1979). Insecticides have also been found in the cord blood of human newborns even when their mothers have no history of occupational exposure to these agents (Bažulić et al, *Bull Environ Contam Toxicol* 32:265, 1984). A large number of studies have identified insecticide toxicity in bird embryos. Many of these reports are based on application of insecticides to the egg shell or direct inoculation of the embryo. Such studies may be important in assessing the environmental impact of these chemicals; however, they do not take into account the considerable biotransformation of these agents by the mammalian mother.

Several rodent species demonstrate an increase in congenital anomalies after exposure to organochlorine insecticides including aldrin, dieldrin, endrin, chlordecone, endosulfan, mirex, and toxaphene (Ottelenghi et al, *Teratology* 9:11, 1974; Gupta et al, *Acta Pharmacol et Toxicol* 42:150, 1978; Chernoff and Carver, *Bull Envir Contam Toxicol* 15:660, 1976; Chernoff and Rogers, *Toxicol Appl Pharmacol* 38:189, 1976). Not all rodent species show effects with these agents and in many cases the "terata" produced consist of minor skeletal anomalies such as supernumerary ribs or a decreased number of ossification centers. One of the best documented reproductive effects of organochlorine insecticides is the production of cataracts in newborn rats associated with feeding mirex to the dam. The cataractogenic effect of mirex may be mediated by fetal hypoglycemia secondary to decreased hepatic glucose-6-phosphatase activity (Rogers et al, *Environ Res* 34:155, 1984).

Teratogenicity tests of the organophosphates have been generally negative; however, some compounds (eg, trichlorfon) in high doses produce anomalous fetuses and a number of the organophosphorus insecticides give rise to minor skeletal defects and/or impaired fetal survival (Kimbrough and Gaines, *Arch Environ Health* 16:805, 1968; Fish, *Am J Obstet Gynecol* 96:1148, 1966; Khera et al, *Bull Environ Contam Toxicol* 22:522, 1979; Staples and Goulding, *Environ Health Perspect* 30:105, 1979). Carbaryl is the only carbamate insecticide for which there are consistent data on teratogenicity. High doses of this agent cause an increase in fetal death in some species and may cause minor skeletal anomalies in guinea pigs (Robens, *Toxicol Appl Pharmacol* 15:152, 1969; Collins et al, *Toxicol Appl Pharmacol* 19:202, 1971); however, this insecticide does not appear to be a significant teratogen or embryotoxin for most species (Weil et al, *Toxicol Appl Pharmacol* 21:390, 1972). Of historical interest is a group of insecticides derived from phthalimide (chemically related to thalidomide). These agents were shown to be embryotoxic and teratogenic in animals (Fabro et al, *Food Cosmet Toxicol* 3:587, 1966; Robens, *Toxicol Appl Pharmacol* 16:24, 1970).

It is important to consider that the teratology studies performed in mammals involve large doses of toxic chemicals. In almost all studies reporting maternal effects of the experiment, adverse symptoms and/or death occurred in many of the dams. When maternal weight gain is indicated, many studies report a decrease in weight or a failure to achieve normal weight gain, presumably due to decreased food intake. It is very likely that adverse fetal outcomes, including impaired survival, growth failure, and some malformations, are attributable to maternal illness and poor weight gain. Although one study (Staples and Goulding, *op cit*) reported that the teratogenic effects (in this case, of trichlorfon in the mouse, hamster, and rat) were not due to reduced food intake, cholinergic symptoms were prominent in two of the species and several dams died.

**OTHER ADVERSE PREGNANCY EFFECTS** — Organochlorine insecticides have been implicated in undesirable pregnancy outcomes in humans. A comparison of 17 women who delivered prematurely with 10 women who delivered at term showed higher mean serum levels of DDT, lindane, dieldrin, and heptachlor epoxide in the former (Wassermann et al, *Environ Res* 28:106, 1982). The ranges of serum DDT values for the two groups demonstrated considerable overlap, however, and it is not possible to determine the significance of the findings from this small sample. The same group subsequently reported a relationship between missed abortion and serum levels of DDT (Bercovici et al, *Environ Res* 30:169, 1983). Organochlorines have also been reported to be present in higher concentrations in maternal blood, placental tissue, and fetal tissues in instances of spontaneous abortion and premature delivery when compared to normal term pregnancies (Saxena et al, *Toxicology* 17:323, 1980; Saxena et al, *J Anal Toxicol* 5:6, 1981). Other possible differences between study and control pregnancies were not, however, explored.

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**CONCLUSIONS** — Insecticides are a diverse group of chemicals used to control household pests, human disease vectors, and agricultural damage. There are a large number of studies in experimental animals which appear to show that many of these agents are a threat to important reproductive processes including the orderly replication of genetic material, fertility, formation of a normal embryo, and survival of the conceptus. These studies, however, feature the exposure of experimental systems to large doses of insecticides. It remains to be shown that insecticides are consistently capable of adverse reproductive effects at doses comparable to likely human exposures. Human epidemiological studies to date have been few in number and have not investigated the possible teratogenicity of insecticides. The suggestion that serum pesticide levels in the mother are associated with adverse reproductive outcome is intriguing but evidence for this is as yet inconclusive. Some pesticides are known to have estrogenic activity, some inhibit a number of important enzymes, and some are demonstrated mutagens; it is, therefore, entirely possible that there is a human reproductive risk to insecticide exposure. As is true for most potential toxins, the most important determinant of reproductive risk is likely to be the amount (duration and dosage) of exposure. Animal evidence suggests that if insecticides are reproductive toxins, severe poisoning of the mother must occur before such toxicity is demonstrated.

### BOOK REVIEW

**Alcohol and the Fetus, A Clinical Perspective** by H.L. Rosett and L. Weiner, New York, Oxford University Press, 1984, 220 pages. The obstetric and pediatric literature has featured an increasing number of studies on the adverse reproductive effects of ethanol. It is now common for clinicians to be asked whether any amount of ethanol ingestion during pregnancy or lactation can be considered safe. There is no easy answer to this and other concerns of parents who may be drinking or who may have been drinking during critical stages of reproduction. This text by two of the workers of Boston University's prominent Fetal Alcohol Education Program does not purport to give easy answers. It is, instead, a review written especially for the clinician in which the large body of work in the field is summarized in an organized manner. The relatively short length and the simple writing style make this an eminently readable treatment suitable for a few relaxed evenings in an armchair.

The book appropriately begins with a history of ethanol and reproduction, a history which illustrates the difficulty with which researchers have sought to determine the exact nature of ethanol embryotoxicity. There follows a short section on the effects of ethanol on the adult including a brief discussion of endocrine and fertility effects. The bulk of the text, however, deals with the embryo and fetus. There is a simple introduction to embryology and teratology with a review of reports of ethanol associated with spontaneous abortion, stillbirth, and prematurity. Of greatest importance are the chapters on ethanol-associated growth abnormalities, morphologic abnormalities, and disorders of intellect and behavior in the offspring.

The summaries of clinical effects notwithstanding, the reader may find the most useful a chapter entitled "Methodologic Issues". Here the difficulties encountered in ethanol research are frankly discussed. The assessing of drinking patterns, the accuracy of information obtained from heavy drinkers, the effects of the manner in which questions are put to drinkers, and the difficulty in defining a "drink" all interfere with identifying which drinkers may be at risk. For example, some heavy drinkers consider an 8 oz glass full of liquor to be one "drink". Other issues considered in the methodologies chapter include the applicability of animal work, the social and physical covariables associated with heavy alcohol use, and the difficulty in identifying subtle congenital anomalies among the wide range of phenotypes in human populations.

The text is well-indexed and can be used as a quick reference on the fetal alcohol syndrome literature. If time permits, however, this volume is best read start to finish as an orderly and clear summary of what has become a large and complex area of continuing investigation.

## LETTER TO THE EDITOR — ALTERNATIVE TESTS FOR TERATOGENS

Sir:

Our laboratory has been involved, in the last six years, in developing an alternative test for detecting teratogens. Our choice of biological material rests on the premise that teratogenesis results from perturbations in the processes that control normal development and thus an *in vitro* test that involves embryonic cells and tissues would monitor fundamental aspects of differentiation and morphogenesis. *Drosophila melanogaster* embryonic cell cultures provide such a system since the cells are capable of normal differentiation *in vitro*. The initial endpoints selected for assessing interference with normal differentiation are muscle and/or neuron differentiation (Bourmias-Vardiabasis and Teplitz, *Teratog Carcinog Mutagen* 2:333, 1982). Over 150 chemicals have already been tested and the results obtained from the *Drosophila* assay show a low percentage of false negatives (<10%) when compared to *in vivo* data (Bourmias-Vardiabasis et al, *Teratology* 28:109, 1983; Bourmias-Vardiabasis et al, *Roux's Arch Dev Biol* 192:299, 1983). In a further refinement of the assay, to include a microsomal fraction for activating proteratogens, we have successfully isolated an S-27 *Drosophila* microsomal fraction capable of activating cyclophosphamide and other proteratogens (Bourmias-Vardiabasis and Flores, *Teratog Carcinog Mutagen* 3:255, 1983). We are also in the process of extending the type of endpoints utilized by including biochemical assays to determine levels of *Drosophila* neurotransmitters after exposure to teratogens (unpublished). Finally in an effort to examine events at the molecular level, we have described the induction of a set of proteins induced after exposure to a variety of teratogens (Buzin and Bourmias-Vardiabasis, *Proc Natl Acad Sci* 81:4075, 1984). The connection between teratogenesis and the induction of heat shock proteins has yet to be determined, but it provides a rather useful means by which one can study the mechanisms controlling embryonic gene expression.

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