for the CFH system (Fig. 1A) the ratio is ~ 2. This photoreaction can be interpreted as an analog of photosynthesis; the reduced compound is molecular hydrogen and the source of electrons is water. However, the stoichiometric ratio of the CFH system can vary widely, depending on reaction conditions, and the ratio of H_2 to O_2 can greatly exceed 2 (*19*). Conversely, Fig. 1B shows that the ratio of H_2 to O_2 for steady-state yields in *Chlamydomonas* is generally < 2. Evidently, under these experimental conditions, not all reducing equivalents are taken up by hydrogenase and evolved as molecular hydrogen (*15*).

Measurements of the simultaneous photoproduction of hydrogen and oxygen have been relatively few compared to those of hydrogen production alone. Spruit (*3*) developed a novel two-electrode polarographic technique for the simultaneous measurement of photoproduced hydrogen and oxygen by *Chlorella*. His principal conclusion was that hydrogen and oxygen metabolisms are closely related and both gases are ultimately given off during illumination from the same source, water. Bishop and Gaffron (*20*) found that the light-dependent evolution of hydrogen appeared to require both photosystems. Gaffron and Rubin (*9*) postulated that the substrate was an organic donor, since addition of glucose caused an increase in the amount of hydrogen evolved [see also (*21, 22*)]. Bishop *et al.* (*23*) used a two-electrode polarographic technique to measure the amount of gas produced in a confined volume; they concluded that water is the primary substrate for hydrogen and oxygen production.

Formidable scientific and engineering development problems remain to be addressed before energy-related applications of photosynthetic hydrogen and oxygen production are possible. These include prevention of wasteful back reactions and development of methods of prolonging the life of the functional components. Moreover, as pointed out by Shinnar (*24*), serious engineering limitations must be dealt with, such as the irreversibilities associated with the production and separation process. The irreversibility loss can be no smaller than the entropy change associated with the process, multiplied by the ambient temperature. Real-world processes will have to cope with fundamental thermodynamic constraints such as these.

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30 July 1981

Prenatal Exposure to the Herbicide 2,4-Dichlorophenyl-*p*-Nitrophenyl Ether Destroys the Rodent Harderian Gland

Abstract. *Exposure of mice to the herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether during gestation produces abnormalities that are not readily apparent at birth but become obvious as the pups mature. By 2 weeks after birth there are severe intraorbital defects resulting from destruction of the Harderian glands behind the eyes. This effect is noticeable only postnatally because the Harderian gland does not grow or function until after birth.*

The preemergence herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether (TOK) (*1*) is used in the cultivation of grain and vegetable crops. Although this compound is relatively nontoxic to adult rats, exposure during gestation to doses two to three orders of magnitude below the median lethal dose (~ 1 g/kg) reduces survival after birth and causes abnormal lung and heart development and diaphragmatic hernias (*2*). Mice surviving such exposure develop a variety of abnormalities, including hydrocephaly, hyperactivity, malocclusion of the jaws, and reduced palpebral fissures (*3*). In addition, they appear to be micro- or anophthalmic (*3*). However, while TOK produces abnormalities and death in mice postnatally, it does not kill fetuses or give them noticeable orbital defects; nor does it induce many obvious abnormalities in neonates (*4*).

In most animal studies of chemical teratogenicity, the dams are killed just before parturition and the fetuses are removed and examined. We suggest that this procedure cannot provide a complete and accurate assessment of the teratogenicity of chemicals like TOK, since they exert their strongest effects postnatally. The present study was designed to examine the apparent micro- and anophthalmic effects of TOK and to determine how such effects elude detection by standard teratological testing procedures.

Primiparous CD-1 mice were randomly assigned to control or experimental

0036-8075/82/0115-0293$01.00/0

Table 1. The postnatal effects of exposing CD-1 mice to TOK during gestation. The values are means ± standard errors. Numbers in parentheses are number of observations (individuals or litters).

Measure	TOK treatment	
	No	Yes
Litter size on day 3	10.8 ± 0.3 (43)	5.4 ± 0.8* (41)
Palpebral height (mm)	2.84 ± 0.15 (5)	2.19 ± 0.17† (10)
Weight of one eye (mg)	25.9 ± 0.6 (37)	25.9 ± 1.7 (31)
Weight of one Harderian gland (mg)‡	18.3 ± 0.7 (29)	15.2 (2)
Body weight of males on day 110 (g)	39.8 ± 0.9 (27)	34.6 ± 0.7* (29)

*Significantly different from control value at $P < .01$ (one-way analysis of variance). †$P < .05$. ‡If present. Paired glands were present in 29 of 29 control animals and in 1 of 30 TOK-treated animals ($P < .01$, chi-square test). One of 30 TOK-treated animals had a single gland behind one eye.

groups. The experimental animals were given TOK (100 mg/kg per day in 0.5 ml of corn oil) by gavage on days 7 to 17 of gestation. Control mice were given vehicle alone. The dams were allowed to give birth, and the pups were counted 3 days later. At 3, 8, and 13 days of age, and as adults, control and treated mice were examined for defects, killed, fixed in Bouin's solution, embedded, and sectioned through the eyes for histopathological evaluation of the Harderian glands and eyes by light microscopy. At 110 days of age, male and female mice from each group were killed with CO_2. The height of the palpebral fissures and the weight of the body, eyes, and Harderian glands were then determined.

The age at eye opening was delayed in the TOK-treated group. Approximately 10 percent of the treated mice did not open their eyes by 30 days of age, while all the control mice opened their eyes by 16 days of age. The eyes of the mice in the TOK-treated group looked small since the height of the palpebral fissures was reduced, but mean eye weight was not reduced (Table 1). In fact, three of the treated mice had eyes that were twice as large as a normal eye (26 mg), the largest eye being 58 mg. It became evident that the apparent microphthalmia was due to an absence of Harderian glands in most of the TOK-treated mice. This caused the eyes to sink deeply into the orbits, narrowing the palpebral fissures. Our microscopic examination of the development of the Harderian glands in control and treated neonatal mice indicated that, although the TOK-treated mice lacked glands, the external appearance of the newborn pups was not altered. The glands initially are so small that they are barely detectable even in controls, but as they grow they cause the eyes to bulge.

A similar experiment was then performed on rats. Pregnant Sprague-Dawley CD rats (Charles River) were given TOK (12.5 mg/kg per day in 0.2 ml of corn oil) by gavage on days 8 to 16 of gestation (*5*). Five males from three experimental litters and 14 males from eight control litters were killed at 70 days of age and necropsied. While all 14 control rats had normal, paired Harderian glands, one of the five TOK-exposed rats had no glands, two had a single gland, and the remaining two had paired glands with abnormal secretions, as indicated by the presence of porphyrin rings around the eyes (chromodacryorrhea). The rings fluoresced red in ultraviolet light. Although the Harderian glands were affected, there was no delay in eye opening. All the rats in both groups had open eyes by day 17.

The Harderian glands are exocrine glands located behind and around the eyes (within the orbits) of reptiles, birds, and mammals that have a nictitating membrane. They are rudimentary or lacking in man (*6*). In the rat, mouse, and hamster, the Harderian glands are large, bilobate, compound tubuloalveolar glands. The secretion of these glands empties onto the inner surface of the nictitating membrane, and its primary function is protection and lubrication of the cornea. The numerous secondary functions vary widely among species. In rodents, a few of these additional functions include secretion of pheromones (*7*), cushioning of the eye, and, in the neonatal rat, possible involvement in extraretinal photoreception (*8*). Harderian gland secretions coat the hair and facilitate thermoregulation in gerbils (*9*).

The TOK-induced destruction of the Harderian glands is easily indentifiable after eye opening but cannot be detected in the fetus or newborn without histological examination because the glands are very small and inactive until the animal is 12 to 13 days old (*10*), at which time they grow rapidly and begin their secretory processes. In the rat, growth of the Harderian glands after eye opening is correlated ontogenetically with an increase in thyroxine secretion (*11*). Thyroidectomy causes Harderian gland regression (*12*), and treatment of neonatal animals with thyroxine accelerates development of the gland (*10*). In light of the influence of thyroid hormones on these glands, it should be noted that TOK and thyroid hormones are all diphenyl ethers and that exposure to TOK lowers serum thyroxine levels (*13*).

The present study demonstrates that prenatal exposure of rats and mice to TOK produces gross morphological defects that usually are apparent later in life, not at birth. One of these defects, the apparent microphthalmia, is not apparent until the Harderian glands enlarge and begin their secretory processes near the time of eye opening. We conclude that, in tests of chemicals like TOK, it is important to examine exposed animals both pre- and postnatally (*14*).

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15. This report was approved for publication by the Health Effects Research Laboratory of the Environmental Protection Agency.

22 June 1981; revised 21 September 1981