Vapor Recovery Systems Can Reduce Risks from MTBE

I would like to comment on the article on methyl tertiary butyl ether (MTBE) in the October issue of *Environmental Health Perspectives* [105:1042–1043 (1997)].

After serving on a panel convened by the U.S. EPA on epidemiological studies needed for evaluation of possible health risks of MTBE as a fuel additive, I feel that the article is quite informative and will no doubt be useful for those contemplating further use of oxygenated fuels. There are two matters that I feel are of some importance, and about them the article is silent.

First, the report of symptoms from use in Fairbanks, Alaska, which stimulated much interest and concern, was associated with two unusual circumstances. First, because of the extreme cold weather, vehicle use is aberrant during the winter in Fairbanks; for example, car motors are left running once they are started. Secondly, the price of fuel increased sharply with the introduction of MTBE, so the public had to realize that there was a change.

The second matter was made clear to the panel, but not widely disseminated. It consisted of the protection provided to drivers and service station attendants by the proper use of approved vapor recovery systems during the filling of gasoline tanks. Because such systems also prevent exposures to other fuel ingredients, which may include a certain amount of benzene, for example, they have an added value in health protection from whatever hazards exposure to gasoline may produce. In addition, they also prevent the emission of hydrocarbons, which can be involved in photochemical pollution. Your illustration of filling a gas tank without an approved vapor recovery system is most inappropriate. The article's discussion of cancer risk also omits the reference to the protection such systems may provide.

I have advised the EPA that the universal requirement of such vapor recovery systems would have several other benefits to health and the environment, as well as obviating much of the need for further epidemiological studies of cancer risks from MTBE. There is broad experience with such systems, and there are abundant measurements to support this position.

John R. Goldsmith
Ben Gurion University of the Negev
Beer Sheva, Israel

Endocrine Disruptors and Testis Development

There is currently much debate as to which *in vivo* tests should be selected for the detection of adverse effects of endocrine disruptors in test animals. As coauthors of a much-cited article in *Environmental Health Perspectives* (*1*), which described small (but significant) decreases in testicular weight of adult rats that had been exposed developmentally to either of two environmental estrogens, we would like to bring certain of our experiences to the attention of readers of *EHP* and to those involved in framing and implementing regulatory guidelines in this area.

In our original paper, we described small but repeatable effects of gestational and lactational exposure to octylphenol (OP) or butyl benzyl phthalate (BBP) on adult testicular weight and daily sperm production in rats. Two subsequent studies by others (*2,3*), both of which were more detailed than our own, have failed to find any effect of BBP, despite using very similar or identical protocols to those used by us. We were asked, and agreed, to help in the design of both of these studies, and we are convinced that every effort was made to truly replicate our original experiments, including the inclusion of a positive effect control group exposed to diethylstibestrol (DES). Though none of us involved in these studies is able to offer an explanation for the differences in results obtained, the inconsistency in effect of BBP in these studies should at least give us all pause for thought. We would like to add to this by reporting other recent experiences of ours that we think have an important bearing on the reproducibility of results based on measurement of adult testis weight and the design and interpretation of any test using this endpoint as an indicator of an adverse effect of developmental exposure to hormonally active chemicals.

Approximately 9 months after completion of our study that was published in *EHP*, testicular weights and body weights in control (untreated/vehicle-treated) animals in our breeding colony began to decline over a period of months. eventually reaching a nadir when the mean ± standard deviation (SD) of testis weight for 36 litters was 1,828 ± 121 mg, compared with the figures of 1,968 ± 163, 2,014 ± 155, and 1,954 ± 118 mg reported in the three experiments in our earlier study (*1*). This temporal decrease in absolute testis weight in control animals was of comparable magnitude to the most severe treatment effect reported in our paper in *EHP* (1,750 ± 180 and 1,847 ± 157 mg in Studies 2 and 3, respectively) (1), which resulted from developmental exposure to DES that had been used as a positive effect control. The decrease in testis weight in controls over time is unexplained, but did follow a permanent change in water supply to our animal facility. In the last 18 months or so, testicular weights in control animals in our colony have returned progressively toward the original values and have now stabilized at levels comparable to those we described (*1*) (1,956 ± 124 mg in the most recent 29 litters).

During the period when control testicular weights were low, developmental exposure of rats to OP (1 mg/l drinking water), using an identical protocol to that used in Study 3 in our earlier study (*1*), failed to cause any significant decrease in testis weight; indeed, weights were increased by 7% (1,824 ± 79 mg in controls, n = 7 litters; 1,950 ± 173 mg in OP-treated, n = 15 litters; $p<0.1$ >0.05). In a subsequent study undertaken when control testis weights had recovered, we were able to confirm a significant effect of DES (50 µg/l drinking water) on absolute testis weight (1,903 ± 146 mg, n = 10 DES-exposed litters; 2,050 ± 84 mg, n = 12 control litters; $p<0.01$) although when expressed relative to body weight, this difference disappeared completely (DES: 4.52 ± 0.30 mg/g body weight; Controls 4.51 ± 0.22; p >0.1). This again contrasts with our original study (though this used 100 µg/l DES). A similar divergence in effect of DES on absolute and relative testis weights was also found in the study by Nair and Jekat (*3*), whereas Ashby et al. (*2*) found a significant reduction in both absolute and relative testis weights after developmental exposure to DES. This inconsistency in the effects of DES occurred despite the fact that all three studies used the same dose level (50 µg/l) of DES. These findings add to growing awareness that endocrine disruption data may be difficult to reproduce in different laboratories or between different studies (*4*), although the reason for these inconsistencies remains obscure.

We are unable to offer an explanation for either the fall/recovery in testicular weights in our control animals or for our failure to obtain similar effects on testis weight/relative testis weight after exposure to OP, and possibly even DES, as we reported originally. We remain confident in the validity of our original published observations, which we consider to fulfill all fundamental scientific criteria with regard to numbers of animals, repeatability of findings, recording

of data, statistical analysis, etc. However, we now consider that biological factors, of which we are unaware and for which we have not controlled, have the potential to exert developmental effects on testis weight which are at least as great as the maximum effects that can be induced by the addition of a potent estrogen (DES) to the mother's drinking water during pregnancy and lactation. This conclusion, and our other experiences outlined above, have obvious relevance to the ongoing debate regarding the design and application of *in vivo* tests for the detection of adverse effects of hormone disruptors. We consider it our scientific responsibility to bring these matters to the attention of all those involved in this area.

Some of the results summarized in the letter above were part of work supported by contract BMH4-CT96-0314 from the European Union.

Richard M. Sharpe
Katie J. Turner
MRC Reproductive Biology Unit
Edinburgh, Scotland

John P. Sumpter
Department of Biology and Biochemistry
Brunel University
Uxbridge, United Kingdom

References

1. Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP. Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environ Health Perspect 103:1136–1143 (1995).
2. Ashby J, Tinwell H, Lefevre PA, Odum J, Paton D, Millward SW, Tittensor S, Brooks AN. Normal sexual development of rats exposed to butyl benzyl phthalate from conception to weaning. Regul Toxicol Pharmacol 26: 102–118 (1997).
3. Nair R, Jekat F, personal communication.
4. AshbyJ, Elliott BM. Editorial: Reproducibility of endocrine disruption data. Regul Toxicol Pharmacol 26: 94–95 (1997).

Authors' note: We are saddened and dismayed to report that the contents of this letter have been communicated without our authority to various sections of the media (Endocrine/Estrogen Newsletter) or in reports being circulated within industry (e.g., by the Chlorine Chemistry Council). These breaches occurred prior even to acceptance of our letter for publication. To add insult to breach of authority, these reports misrepresent our letter as a retraction of our original findings. This is not the case, as anyone who reads the letter above can confirm.

The First Synthetic Estrogen

In the course of a literature review, I encountered a report published in 1933, which described the first synthetic estrogen (*1*). At that time, an incorrect version of the chemical structure of estrone was in use, but the first synthetic estrogen was derived from it, namely, 1-keto-1,2,3,4-tetrahydrophenanthrene. Estrogenicity was demonstrated by changes in vaginal cytology in ovariectomized rats. Two parts of the discussion section of the paper are beautiful to read, as follows:

> This result is of importance, for 1-keto-1,2,3,4-tetrahydrophenanthrene is the first compound of known chemical constitution found to have definite oestrus-exciting activity. There is thus provided the first step in the task of defining the molecular conditions necessary for this type of physiological activity, and there are grounds for hoping that substances of a much higher order of activity will be found before very long. . . .
>
> The observation that oestrogenic properties of a low order are possessed by suitable extracts of such a variety of materials as peat, brown coal, lignite, coal tar and petroleum is of interest, but in view of the fact that many such materials are known to contain carcinogenic constituents, the clinical use of such extracts without very stringent refinement is scarcely to be entertained.

This seminal paper therefore mentions synthetic estrogens, a test for estrogens, hopes for structure–activity relationships among estrogens, naturally occurring estrogens, the anticipated clinical application of estrogens, and a relative risk estimate, with carcinogenicity being weighed against estrogenicity. Within 3 years, the same group had defined bisphenol A as an experimental estrogen (*2*). Sixty years later, the United States Congress mandated an ordered study of synthetic environmental estrogens (*3*).

John Ashby
Zeneca Central Toxicology Laboratory
Alderley Park
Cheshire, United Kingdom

References

1. Cook JW, Dodds EC, Hewett CL. A synthetic oestrus-exciting compound. Nature 131:56–57 (1933).
2. Dodds EC, Lawson W. Synthetic oestrogenic agents without the phenanthrene nucleus. Nature 137:996 (1936).
3. Food Quality Protection Act of 1996. 104th Congress, 2nd Session,1996.