Endosulfan's Effects: Omissions and Flawed Data

The main objective of the Endosulfan Manufacturers and Formulators Welfare Association in India is disseminating scientific facts concerning the use of endosulfan. Because endosulfan is used in several countries, the association watches for global news and information relevant to endosulfan.

Members of the Endosulfan Manufacturers and Formulators Welfare Association are of the strong opinion that Saiyed et al. (2003) omitted significant information in their published report "Effect of Endosulfan on Male Reproductive Development." Consequently, to uninitiated readers the article presented an alarming picture of endosulfan, a pesticide registered for use in over 70 countries for more than 40 years.

Saiyed et al. (2003) failed to mention the origin of their study. In fact, their study is an integral part of an epidemiologic study submitted to the government of India in 2003, "Report of the Investigation of Unusual Illnesses Allegedly Produced by Endosulfan Exposure in Padre Village of Kasargod District" [National Institute of Occupational Health (NIOH) 2003]. After studying this report and other related studies, an expert group appointed by the government of India categorically concluded in February 2003 that "there is no link established between use of endosulfan in PCK [Plantation Corporation of Kerala] plantations and health problems reported in Padre village" (Central Insecticides Board and Registration Committee 2003). The government of India has since accepted this conclusion.

Epidemiology is a science of proving association and not causation. Therefore, we consider the conclusion of Saiyed et al. (2003) that their "study results suggest that endosulfan exposure may delay sexual maturity and interfere with hormone synthesis in male children" to be unscientific, uncalled for, and objectionable.

Saiyed et al. (2003) failed to mention the fact that, besides endosulfan, a host of other pesticides were also used in both exposed and control study areas. The comment by *EHP*'s Science Editor, Jim Burkhart, in the press release dated 1 December 2003 (EHP 2003) that "decades of spraying this pesticide [endosulfan], and only this pesticide" is therefore erroneous.

The authors failed to mention the actual quantity of endosulfan aerially applied in the cashew plantation block closest to Vaninagar school (exposed group), which was 105 g active ingredient (ai)/acre/year. This rate should be compared with the permissible seasonal application rates elsewhere, including the United States, where 1,000 g ai/acre/year is often exceeded.

Saiyed et al. (2003) failed to take into consideration the fact that the interval between annual aerial applications was nearly 11 months and that the aerial applications were made 5–6 months ahead of monsoon rains. All this must be weighed against the known half-life of endosulfan in conditions in India (30–50 days).

In the proceedings of the expert group, the experts on epidemiology and residue science raised serious objections to the study design, sample selection, and residue analysis adopted by the NIOH (2003).

Endosulfan residue levels reported by Saiyed et al. (2003) in blood samples are 1,000 times greater than the residue levels reported in water samples by the authors in the original report submitted in India (NIOH 2003). The scientific properties of endosulfan do not support this phenomenon. Also, two studies by Kerala Agricultural University (State of Kerala 2001) that preceded the article by Saiyed et al. (2003) did not find endosulfan residues in water in Padre village.

These are a few representative omissions and flaws in the article by Saiyed et al. (2003). Knowing the reputation and professional excellence of *EHP,* I appreciate the chance to address these problems here.

The author declares a competing financial interest because he is retained as executive secretary to the Endosulfan Manufacturers and Formulators Welfare Association of India.

C. C. Abraham
Endosulfan Manufacturers and Formulators Welfare Association
Mumbai, India
E-mail: ccabraham69@hotmail.com

References

Central Insecticides Board and Registration Committee. 2003. Minutes of the 233rd Meeting of the Registration Committee. Available: http://www.cibrc.nic.in/rc.htm [accessed 16 April 2004].

EHP. Young Males Exposed to Pesticide Endosulfan See Delay in Sexual Maturation. Research Triangle Park, NC:Environmental Health Perspectives, National Institute of Environmental Health Sciences. Available: http://ehp.niehs.nih.gov/press/120103.html [accessed 8 January 2004].

NIOH. 2003. Investigation of Unusual Illnesses Allegedly Produced by Endosulfan Exposure in Padre Village of Kasargod District (N. Kerala). Ahmedabad, India:National Institute of Occupational Health.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H, et al. 2003. Effect of endosulfan on male reproductive development. Environ Health Perspect 111:1958–1962.

State of Kerala. 2001. Report on the Visit of the Expert Team Constituted for Investigating the Environmental Effects of Aerial Sprayed Endosulfan in Perla Area of Kasargod District (19 February 2001). Trivandrum, India:State of Kerala.

State of Kerala. 2001. Report on the Visit of the Expert Team Constituted for Investigating the Environmental Effects of Aerial Sprayed Endosulfan in Kasargod District (August 2001). Trivandrum, India:State of Kerala.

Endosulfan's Effects: Inaccurate Data

We would like to address problems with the article "Effect of Endosulfan on Male Reproductive Development" by Saiyed et al. (2003). Our comments are based on extensive discussions with several experts and decision-making bodies on the subject.

First, Saiyed et al. (2003) described the results from a report first submitted in India in July 2002 by the National Institute of Occupational Health (NIOH 2002). We noted with interest that Saiyed et al. (2003) did not include additional or new data in the article beyond what was presented in the original report (NIOH 2002). Consequently, there was no need for discussing the results again.

The original NIOH report (NIOH 2002) was submitted to an expert panel of Indian scientists appointed by the Government of India. The results of this review were reported to the Indian Central Insecticides Board and Registration Committee in April 2003. The committee was highly critical of the overall conduct of the study and concluded that "there is no link established between use of endosulfan in PCK [Plantation Corporation of Kerala] plantations and health problems reported in Padre village"(Central Insecticides Board and Registration Committee 2003). This was further endorsed at a subsequent meeting of the Registration Committee in September 2003. The Indian scientists who reviewed this report evidently had some major advantages of additional information that were not available to the reviewers of the article by Saiyed et al. (2003). Namely, they had access to all of the data, they could consult the authors, and they clearly comprehended the local situation in the region of India under consideration. Therefore, they found that the NIOH study (NIOH 2002) failed to prove what appeared to be an apparently flawed hypothesis.

Referring to the statement by the Central Insecticides Board and Registration Committee (2003), the Minister for Agriculture reiterated in the Indian parliament that "The [Government] of India also constituted an expert committee and based on its recommendations decided that the use of endosulfan be continued as per provisions of Insecticides Act 1968 as there is no link established between the use of endosulfan in PCK plantations and health problems in Padre village."

In their article in *EHP*, Saiyed et al. (2003) emphasized the premise that the geographical area studied was unique, with only a single pesticide used over a long period of time. Unfortunately, this is not correct; local records show that several pesticides were applied in this area. Furthermore, what is

also apparent from local records is that several of the same pesticides were also commonly used in the control area. Thus, the scientific value of this study is questionable.

For sexual maturity rating (SMR) and hormone levels, the results presented by Saiyed et al. (2003) displayed a relatively poor correlation with age; this is not enough to clearly describe a positive correlation. There are two additional confounding factors: *a*) small sample size (small number of subjects and only one blood sample per subject), which was recognized by the authors, and *b*) Saiyed et al. (2003) did not mention the normal biological ranges for either SMR or hormone levels in such a population. For example, serum hormone levels are highly variable and, without reference to what would be considered a normal range, the authors cannot confidently claim that the apparent changes they described were caused by pesticide exposure. This is evident in the last paragraph of the article, in which Saiyed et al. (2003) concluded that " long-term follow-up of the children is essential to understand the implications." As a measure of endosulfan exposure, the authors described serum levels of endosulfan and endosulfan sulphate. There are a number of interesting paradoxes with these results. The first is that endosulfan was not used in the area, as described in the article, for 10 months before the study started. Given the rapid biodegradation and subsequent clearance of endosulfan in this type of environment, it is surprising that endosulfan was discovered in these samples. To further confound this, the levels of endosulfan, again as described by Saiyed et al. (2003), were ≥ 0.03 ppb in water and ≥ 0.3 ppb in pond sediments, well below the levels in the serum samples. Endosulfan is rapidly cleared from the body and does not bioaccumulate; thus, the serum levels are clearly at odds with those found in water samples, including those of the control samples. It should also be added that the water levels of endosulfan described by Saiyed et al. (2003) are well below the maximum recommended by the U.S. Environmental Protection Agency (EPA).

The distance between PCK plantations and Vaninagar school is about 3 km. The amount of endosulfan applied in the PCK plantation block closest to Vaninagar school was about 105 g active ingredient (ai)/acre/year, whereas the permissible application rates authorized by the U.S. EPA are > 1,000 g ai/acre/season. Saiyed et al. (2003) did not include this fact in their article.

In the area described by Saiyed et al. (2003), aerial application was carried out by helicopter at a height of about 10 feet above cashew tree plantations. It is highly unlikely that the sprayed endosulfan would drift away all the way to the Vaninagar school area 3 km away. Also, the interval between annual aerial applications was about 11 months. Endosulfan is likely to undergo significant degradation during this period.

In this area, it is highly unlikely that significant amounts of endosulfan would have translocated with rainwater runoff during monsoon rains, which normally occur 5–6 months after aerial application. Under typical conditions in India, significant degradation of endosulfan would have occurred.

The endosulfan residue levels Saiyed et al. (2003) reported in blood samples were 1,000 times higher than those reported in water samples. Based on the physicochemical properties of endosulfan, such a finding is highly unlikely.

Finally, the reproductive effects of endosulfan reported by Saiyed et al. (2003) are inconclusive.

We are concerned with the comment made by *EHP*'s Science Editor, Jim Burkhart, in the press release dated 1 December 2003 (EHP 2003):

> Decades of spraying this pesticide, and only this pesticide, on the village provided a unique opportunity to analyze its impact. Although the sample size is somewhat limited, the results are quite compelling.

Calling the results "compelling" has caused significant paranoia among users of endosulfan and the general public. A number of pesticides were in use around Padre village for many decades, so it was not correct to state that only one pesticide was used.

In conclusion, we hope we have shown that the article by Saiyed et al. (2003) does not contribute to the knowledge on the behavior of endosulfan or to the research to determine the cause of health problems of the people in Padre village.

In view of the foregoing facts, we believe that *EHP* should reevaluate their review of the article by Saiyed et al. (2003).

The author is employed by CropLife India, an organization that represents the plant science industry.

A. S. Indulkar
CropLife India
Mumbai, India
E-mail: croplifeind@vsnl.net

References

Central Insecticides Board and Registration Committee. 2003. Minutes of the 233rd Meeting of the Registration Committee. Available: http://www.cibrc.nic.in/rc.htm [accessed 26 May 2004].

EHP. 2003. Young Males Exposed to Pesticide Endosulfan See Delay in Sexual Maturation. Research Triangle Park, NC:Environmental Health Perspectives, National Institute of Environmental Health Sciences. Available: http://ehp.niehs.nih.gov/press/120103.html [accessed 6 January 2004].

NIOH. 2002. Investigation of Unusual Illnesses Allegedly Produced by Endosulfan Exposure in Padre Village of Kasargod District (N. Kerala). Ahmedabad, India:National Institute of Occupational Health.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H, et al. 2003. Effect of endosulfan on male reproductive development. Environ Health Perspect 111:1958–1962.

Endosulfan's Effects: Saiyed's Response

I would like to respond to the comments of both Abraham and Indulkar about the origin and results of our study (Saiyed et al. 2003).

On the basis of media reports of unusual illnesses in Padre village, we were asked by the National Human Rights Commission to investigate these illnesses and determine if they were linked to endosulfan exposure. These reported illnesses consisted of birth defects, growth- and development-related problems in children, neurologic disorders, epilepsy, allergic disorders, cancers, and high rates of suicide. On the basis of our site visit, study of the topography of the area, the report of the Regional Remote Sensing Service Centre (Nageswara Rao PP, personal communication), and information from the Plantation Corporation of Kerala (PCK), we determined that we had a unique opportunity to study various health effects of long-term exposure to endosulfan on human health.

Our study (Saiyed et al. 2003) had two components: *a*) an investigation of growth- and development-related parameters, such as physical growth (height and weight), skin-fold thickness, IQ, study of behavioral problems and scholastic performance, sexual maturity rating (SMR), and an estimation of sex hormones, in schoolchildren exposed to the aerial spray of endosulfan; and *b*) an investigation of diseases, such as neurologic and psychiatric disorders, infertility, and allergic disorders, in the adult population, which was carried out through secondary data collection from parents of the children.

In our report submitted to the National Human Rights Commission, [National Institute of Occupational Health (NIOH) 2002], we reported a higher prevalence of birth defects in both male and female children, lower mean SMR and serum testosterone levels in male children of similar age, a higher prevalence of scholastic backwardness, and neurobehavioral problems in children who had been exposed to endosulfan through aerial treatment of fields (NIOH 2002). We suggested a possible link between endosulfan exposure and these problems, which has been reported in a number of published animal experimental studies, for example, endosulfan-related birth defects (Food Machinery and Chemical Corporation 1980;

García-Rodríguez et al. 1996; Gupta et al. 1978]; scholastic development (Lakshmana and Raju 1994; Paul et al. 1994); and reproductive effects in males (Dalsenter et al. 1999; Sinha et al. 1997, 2001). We submitted our report to the Registration Committee at their request. The eight-member expert committee, which included two major stakeholders of endosulfan in the country, was set up by the Registration Committee to examine our report along with the report of the Kerala Agriculture University (KAU) and the Fredrick Institute of Plant Protection and Toxicology (FIPPAT). The committee raised issues, many of which are also mentioned in the letters from Abraham and Indulkar, and remarked that "the findings of the NIOH study are not in conformity with the known and accepted properties, chemistry and toxicology of endosulfan" (Dubey 2003). The committee concluded that the study did not establish a link between endosulfan exposure and the health problems in Padre village. The decision of the committee was not unanimous.

One of the important components of any scientific investigation is dissemination of information to other scientists through publication of the results in a suitable journal; therefore, we submitted a manuscript covering part of the study to *EHP*. The opinion of the expert committee and their deliberations in no way affected our right of scientific communication. Our study was scientifically planned, designed, and carried out by a team of experts, which included epidemiologists, physicians, pediatricians, medical toxicologists, statisticians, analytical chemists, and biochemists, who have years of experience in conducting such studies and have many publications to their credit. Our article (Saiyed et al. 2003) was based on a portion of the results of the study in children. Some of the other parameters related to growth and development will be communicated at a later date.

Both Abraham and Indulkar comment that several pesticides were used in the area, so endosulfan cannot be blamed for the reported health problems. Based on the topographic study of the area, we were concerned about what was sprayed on the cashew plantation, which covered a very large area on the hills; we believed that compounds sprayed on the plantation would run off into streams used by residents of the village downhill from the plantation. We asked the PCK, the owners of the cashew plantation, to give us information on all pesticides that were sprayed in the study area. On 20 August 2001, PCK informed us that since 1980 they had aerially sprayed endosulfan (0.1% of 35% emulsifiable concentrate) twice a year almost every year. They did not mention the use of any other pesticides. In their letters, Abraham and Indulkar mention the use of other pesticides in the area; this probably refers to the valley where there are small farms that are owned by the local families. The major crop in this valley and in the control area is areca nut, for which Bordeaux mixture (copper sulfate and lime) is used. The small family farms in the valley and in the control village had a similar crop pattern; therefore, we can assume localized ground use of pesticides. This type of localized pesticide use is unlikely to cause significant widespread exposure. Our conclusions (Saiyed et al. 2003) were based on the comparison of the control and study areas on the basis of aerial exposure; therefore, the use of other pesticides, if similar in both the study and control areas, would not affect our conclusions. The issue of finding endosulfan in serum samples of the control population was adequately addressed in the "Discussion" of our article (Saiyed et al. 2003).

In their letters, Abraham and Indulkar comment that downward movement of the pesticides could start only at the onset of monsoons 5–6 months after exposure, by which time no endosulfan would remain because it biodegrades rapidly. The NIOH and three other agencies carried out endosulfan analyses at varying times (January 2001–August 2001) and reported significant amounts of endosulfan in various environmental media. First, the Centre for Science and Environment, New Delhi (a nongovernment organization), analyzed biological and environmental samples for endosulfan residues in January 2001, 1 month after the last aerial spray of endosulfan carried out on 26 December 2000. The results showed that the concentration of endosulfan in three water samples was 7–51 times higher than the maximum residue limit (MRL) (Joshi 2001). Very high levels of endosulfan were reported in samples of human blood, human milk, vegetables, spices, cow's milk, animal tissues, cashews, cashew leaves, and soil. In one of the soil samples, the concentration of endosulfan was 391 times higher than the MRL.

Second, the KAU, Thrissur District (Kerala), India, studied endosulfan levels in soil (n = 4), plants (n = 5), water (n = 5), and sediment (n = 1) in the pond in the valley on 19 February 2001 using an HPLC–spectrophotometric detector technique. They reported endosulfan in soil (3,815 ppb on PCK plantation), 55 ppb in the mid-hills, and 315 ppb in the sediment in the pond (Mathew S, personal communication). They also reported 507–858 ppb endosulfan in cashew leaves. Their report clearly demonstrated downward movement of endosulfan from hills to the pond water (Figure 1). (Mathew S, personal communication).

Third, FIPPAT, Padappai, India, carried out sampling between March and May 2001 in cashew leaves (n = 28), human blood (n = 112), water (n = 30), and soil (FIPPAT 2001). The authors reported up to 3,430 ppb endosulfan in cashew leaves, 1–11 ppb in soil, and no endosulfan in blood or water samples. On examination of the report (FIPPAT 2001), we noted large peaks of alpha- and beta-endosulfan in some chromatographs of soil, leaf, and blood samples that they had not mentioned. We reported this discrepancy to the expert committee and requested action.

The results of the studies by the Centre for Science and Environment in January 2001 (Joshi 2001), the KAU in February 2001 (Mathew S, personal communication), and FIPPAT (2001) in March–May 2001 clearly indicate significant translocation of endosulfan from the hills to the valley and its persistent nature.

Abraham and Indulkar both noted a discrepancy in our article (Saiyed et al. 2003) between endosulfan levels in serum and water. They both assume that the endosulfan exposure occurred only through water, which is not correct. Endosulfan adhers to the soil particles; runoff water then carries the endosulfan attached to soil particles from leaf surfaces and the ground during the first few rainfalls. Also, winter rains are common in this area of India. This view is largely supported by the KAU study (Mathew S, personal communication), which showed high levels of endosulfan (315 ppb) in pond sediment in the study village. Endosulfan attached to soil particles can enter the body

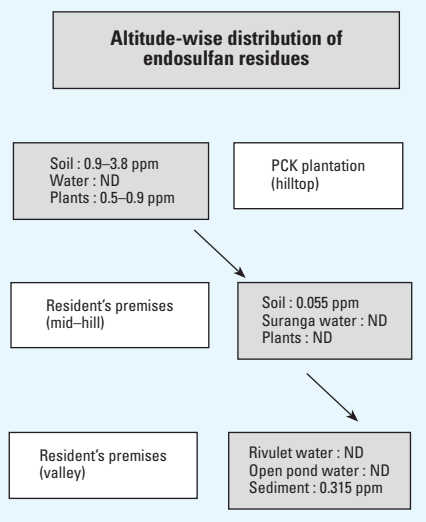

Figure 1. Altitude-wise distribution of endosulfan. ND, not detected. Reproduced with permission from Samuel Mathew, Department of Chemistry, Kerala Agriculture University.

through the dermal route, more so in a hot and humid climate (excessive sweating), and through ingestion, particularly in children, who commonly have hand-to-mouth behavior. Endosulfan attached to the soil particles can also be translocated through other environmental media.

Indulkar raised several points about the results of our article (Saiyed et al. 2003). He incorrectly stated that we found poor correlation of SMR and hormone levels with age; our correlations are shown in Tables 2 and 3 of our article (Saiyed et al. 2003). He also complained that we had not compared the ranges of SMR and hormone levels in our study groups to normal ranges, but we did not consider it necessary because we were comparing the groups with each other. Also, Indulkar's points about the wide variability of hormone levels and small sample size were discussed and clarified in our article.

Both Abraham and Indulkar stated that endosulfan cannot travel a distance of 3–4 km and that it biodegrades quickly. The long half-life of endosulfan in soil is well known and was mentioned in our "Discussion" (Saiyed et al. 2003). The U.S. Environmental Protection Agency (EPA 2002) reported that

> Endosulfan is a volatile and persistent cyclodiene pesticide that can migrate over a long distance through various environmental media such as air, water and sediment. Once endosulfan is applied to crops, it can either persist in soil as a sorbed phase or be removed through several physical, chemical and biological processes. Recent studies suggest that secondary emissions of residual endosulfan continue to recycle in the global system while they slowly migrated and are redeposited via wet deposition in the Northern Hemisphere. The occurrence of endosulfan in remote regions like the Great Lakes, the Arctic and the mountainous areas is well documented. Endosulfan can also enter the air as adsorbed phase onto suspended particulate matter, but this process does not appear to be a major contributor to long range transport like volatilization.

The information provided above further supports the comment made by Jim Burkhart, *EHP* Science Editor, that

> Decades of spraying this pesticide, and only this pesticide, on the village provided a unique opportunity to analyze its impact. Although the sample size was somewhat limited, the results are quite compelling.

We stand by the conclusions in our article (Saiyed et al. 2003).

The author declares he has no competing financial interests.

Habibullah N. Saiyed
National Institute of Occupational Health
Ahmedabad, India
E-mail: saiyedhn@yahoo.com

References

Dalsenter PR, Dallegrave E, Mello JRB, Langeloh A, Oliveria RT, Faqi AS. 1999. Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and lactation. Hum Exp Toxicol 18:583–589.

Dubey OP. 2003. Report of Expert Group on Endosulfan. New Delhi:Pesticide Registration Committee, Ministry of Agriculture, Government of India.

FIPPAT. 2001. Evaluation of Residues of Endosulfan in Human Blood, Cow Milk, Fish, Water, Soil and Cashew Leaves. Report No. 8881; Project No. 04-1962-2001. Kancheepuram District, Tamil Nadu, India:Fredrick Institute of Plant Protection and Toxicology.

Food Machinery and Chemical Corporation. 1980. Final Report: Teratology Study with FMC 5462 in Rats. Raltech study no. 79041. Madison, WI:Raltech Scientific Services.

García-Rodríguez J, García-Martín M, Nogueras-Ocaña M, Luna-del-Castillo JD, García MF, Olea N, et al. 1996. Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ Health Perspect 104:1090–1095.

Gupta PK, Chandra SV, Saxena DK. 1978. Teratogenic and embryotoxic effects of endosulfan in rats. Acta Pharmacol Toxicol 42:150–152.

Joshi S. 2001. A curse called endosulfan: a Kerala village blames aerial spraying of the pesticide endosulfan for unusual diseases. Down to Earth 9(19):28–35.

Lakshmana MK, Raju TR. 1994. Endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. Toxicology 91(2):139–150.

NIOH. 2002. The Investigation of Unusual Illnesses Allegedly Produced by Endosulfan Exposure in Padre Village of Kasargod District (N. Kerala). Ahmedabad, India:National Institute of Occupational Health.

Paul V, Balasubramaniam E, Kazi M. 1994. The neurobehavioural toxicity of endosulfan in rats: a serotonergic involvement in learning impairment. Eur J Pharmacol 270(1):1–7.

Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan R. et al. 2003. Effect of endosulfan on male reproductive development. Environ Health Perspect 111:1958–1962.

Sinha N, Adhikari N, Saxena DK. 2001. Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. Environ Toxicol Pharmacol 10:29–32.

Sinha N, Narayan R, Saxena DK. 1997. Effect of endosulfan on the testis of growing rats. Bull Environ Contam Toxicol 58:79–86.

U.S. EPA. 2002. Re-registration Eligibility Decision for Endosulfan. No 738-R-02-013. Washington, DC:U.S. Environmental Protection Agency.

Defining Wetlands

I read with interest David J. Tenenbaum's article about constructed wetlands in the January 2004 issue of *EHP* (Tenenbaum 2004). While I noted his caveat that "their design remains a bit of an uncertain art," I think he should have gone further to ensure that engineers do not immediately embrace constructed wetlands as a panacea for waste treatment and decontamination.

I also find myself increasingly unsettled by articles such as this one as potentially and inadvertently capitalizing on the opinion of the populace that wetlands are good places—a widespread view these days (deservedly so) because of excellent advocacy by government agencies and environmental nongovernment organizations such as Ducks Unlimited.

Although the sewage treatment facilities promoted in the article (Tenenbaum 2004) may resemble wetlands, they are far from the real thing, or at least as far as the term is generally understood. In fact, I noted that later in the article Tenenbaum turned his attention to wetland restoration projects. In my experience, these again generally involve real wetlands, so to speak. Thus our wetland contrivances, enhancements, or other more utilitarian versions are more fairly not referred to as "restoration" for the straightforward reason that the former condition of these sites, the target for restoration, did not include sewage treatment.

The waste treatment benefits from constructed wetlands are clear, and Tenenbaum (2004) is to be commended for pointing this out as well as the uncertain risks. We cross the line, however, when we pitch constructed wetlands without mentioning the caveat that these surrogate ecologic features are intended to become contaminated (per heavy metal sinks, for example) and that this will impair other uses for these facilities, including use as habitat for species that rely on wetlands and cannot discern the real from the fake. Nature's way advises us to be responsible about our waste generation at the source and not to dress up the end-of-pipe solution.

The author declares he has no competing financial interests.

Harald Schraeder
Biologist
St. Thomas, Ontario, Canada
E-mail: hschran623@rogers.com

Reference

Tenenbaum DJ. 2004. Constructed wetlands: borrowing a concept from nature. Environ Health Perspect 112:A44–A48.

Phthalate Exposure and Early Thelarche

Several years ago Colón et al. (2000) reported higher levels of phthalates, particularly di-(2-ethylhexyl) phthalate (DEHP) in serum from 41 girls experiencing premature breast development (thelarche) as compared to 35 age-matched controls. These data seem puzzling for at least two reasons. First, in light of the pharmacokinetic properties of phthalates, the reported blood levels are very high when compared to more recent exposure information, and, second, toxicologic evidence shows that phthalates do not act like estrogens under *in vivo* conditions, nor do they affect female sexual development and maturation in rodents. Despite these concerns, the study by Colón et al. (2000) is one of the few studies that has compared chemical exposures with sexual development and, as a consequence, it is now being cited by authors developing

hypotheses for future research programs on children's health (e.g., Chapin et al. 2003). There have been a number of recent scientific developments that relate to these matters. Thus, it seemed timely to summarize the exposure and toxicologic information that would be relevant in assessing whether low-level exposure to phthalates, which might be experienced under typical ambient conditions, could influence human female sexual development.

Colón et al. (2000) reported an average DEHP concentration of 450 ppb (nanograms per milliliter) DEHP and 3 ppb monoethylhexyl phthalate (MEHP, the first metabolite of DEHP) in serum samples from the referred cases. In comparison, they found 70 ppb DEHP in controls, whereas MEHP was below levels of detection. In general, the measurement of phthalates in biological fluids and other media can be quite problematic because of the potential for laboratory contamination due to the use of flexible vinyl in laboratory equipment and tubing. Also, the use of flexible vinyl in liners for reagent bottles is common (e.g., Kessler et al. 2001). Much of the historical data on phthalate levels is questionable, and investigators are now measuring phthalate metabolites in biological media as a way of avoiding the sample contamination problems (e.g., Blount et al. 2000a, 2000b; Kessler et al. 2001). However, taking the data at face value, the results are difficult to rationalize with information on phthalate pharmacokinetics and exposure.

Within the population at large, the principal route of exposure to phthalates is via food (e.g., Clark et al. 2003). Ingested phthalates are converted to the corresponding monoesters before absorption (e.g., Kluwe 1982), and blood levels of phthalate diesters are normally very low. To put this into perspective, a blood level of 450 ppb DEHP is approximately 1.2 μM. In a recent pharmacokinetic study of DEHP in the marmoset (Kessler et al. 2004), oral doses of DEHP at levels of 30 and 500 mg/kg resulted in peak blood levels of DEHP of 0.3 and 1.8 μM. Using a linear allometric extrapolation, an oral dose of approximately 300 mg/kg DEHP would be required to produce a blood level of 450 ppb. In contrast, the average DEHP exposure among U.S. children at ages similar to those examined by Colón et al. (2000) is 2.6 μg/kg/day (McKee et al. 2004; calculated from urinary metabolite data of Brock et al. 2003). Thus, the blood concentration data reported by Colón et al. (2000) are very unusual, and, if correct, imply extraordinary exposures by comparison to those of other children of similar ages.

Further, as indicated above, DEHP is rapidly metabolized to its corresponding monoester, MEHP [mono-(2-ethylhexyl) phthalate]. In a pharmacokinetic study in marmosets (Kessler et al. 2004), oral doses of 30 and 500 mg/kg resulted in peak MEHP concentrations of 8 and 66 μM, approximately 20 times greater than the peak DEHP levels at equivalent doses. In contrast, the average MEHP level of 3 ppb reported by Colón et al. (2000) was more than two orders of magnitude below the reported average DEHP level. The only imaginable situation that might produce such anomalous results is if the blood samples had been taken immediately after direct introduction of DEHP into the blood stream. This is theoretically possible, but the reported levels still seem unlikely. The only way in which significant levels of unmetabolized phthalates may enter the blood stream is by way of medical devices including blood bags, tubing, and other medical equipment [e.g., Food and Drug Administration (FDA) 2001]. DEHP is the primary phthalate plasticizer for such vinyl medical devices. However, as shown in an assessment by the FDA (2001), only critical-care procedures could produce blood levels that begin to approach the levels reported by Colón et al (2000). Further, in blood, DEHP is rapidly metabolized with a biological half-life in humans of < 6 hr (e.g., Peck and Albro 1982). Thus, assuming medical treatment to be the explanation for the high DEHP/MEHP ratio reported by Colón et al. (2000), the measured blood levels imply intensive care procedures performed in the few hours proceeding the blood collection. However, given the circumstances this seems unlikely.

In the study by Colón et al. (2000), the blood samples were taken from children after referral to pediatric clinics, that is, after premature thelarche had been observed. Because phthalates are rapidly metabolized and excreted, the blood level data, if not the result of laboratory contamination, could only have reflected exposures occurring shortly before the blood was drawn. For the hypothesized link between phthalate exposure and early breast development to be supported, one would have to assume that these point estimates were indicative of a pattern of exposure that had persisted for an extended period of time. But, as shown above, the conditions leading to the reported blood levels are so unusual that they cannot reflect an extended exposure profile. Analytical difficulties seem a more likely explanation to reconcile the data reported by Colón et al. (2000) with the extensive body of information on phthalate pharmacokinetics.

The second general reason why an association between phthalate exposure and the early onset of puberty in girls is puzzling and seems unlikely is that it is not supported by the toxicologic data. There is a hypothesis that premature thelarche is a consequence of unintentional exposure to estrogen or estrogen-like substances (e.g., Li et al. 2002; Tiwary 1998). It has been reported that some phthalates, although not DEHP, can bind and activate the estrogen receptor under *in vitro* conditions (e.g., Jobling et al. 1995; Soto et al. 1995); however, subsequent work has shown that this is an artifact of the testing conditions.

As described above, under *in vivo* conditions the phthalates are rapidly metabolized to the corresponding monoesters, but these metabolites are not active under *in vitro* conditions (Brady et al. 1998; Harris et al. 1997; Picard et al. 2001). In rats and mice, phthalates are inactive in uterotrophic assays and do not induce vaginal cornification (Kanno et al. 2003; Zacharewski et al. 1998). Similarly, in longer-term studies phthalates do not influence age at vaginal patency or estrous cyclicity and do not otherwise influence sexual development or behavior in female rats (Moore 2000). Some phthalates produce effects in male rats, apparently as a result of alterations in testosterone synthesis (Parks et al. 2000), but these alterations do not produce estrogen-like effects in female rats (Gray et al. 1999; Moore et al. 2001; Mylchreest et al. 1998, 1999). With respect to female reproductive development, the only effect that has been described in rats relates to histologic changes in the ovary that occur at very high doses (Lovekamp-Swan and Davis, 2003; Lovekamp-Swan et al. 2003).

These changes seem to be caused by inhibition of aromatase activity in the granulosa cells. Aromatase inhibition reduces conversion of testosterone to estradiol and produces what is effectively an antiestrogenic effect under these circumstances. However, this seems unlikely to have any public health implications, partly because the doses required in rodents are much higher than those to which humans are exposed and also because the aromatase inhibition is dependent on activation of the peroxisome proliferator-activated receptor α (PPARα). In other situations, there is evidence that PPARα-dependent effects exhibit profound species specificity, with rodents being very sensitive and humans being much less sensitive, if not refractory (e.g., Klaunig et al. 2003).

In summary, the purported relationship between phthalate exposure and early thelarche (Colón et al. 2000) seems highly unlikely, in part because the reported exposure levels do not seem plausible given other information on phthalate exposure, and also because phthalates do not influence the timing of female sexual development in laboratory studies.

The author wrote this letter in his capacity as chairman of the Toxicology Research Task Group of the Phthalate Esters Panel, a trade association representing the phthalate industry. He certifies that his freedom to design, conduct, interpret, and publish research was not compromised by any controlling sponsor as a condition of review and publication.

Richard H. McKee
Toxicology Research Task Group
Phthalate Esters Panel
American Chemistry Council
Arlington, Virginia
E-mail: richard.h.mckee@exxonmobil.com

References

Blount B, Milgram K, Silva M, Malek N, Reidy J, Needham L, et al. 2000a. Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. Anal Chem 72:4127–4134.

Blount B, Silva M, Caudill S, Needham L, Pirkle J, Sampson E, et al. 2000b. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 108:979–982.

Brady A, Moffat G, Martens M. 1998. Assessment of in vivo estrogenic activity of butylbenzyl phthalate (BBP) and its metabolites. Toxicologist 42:176–177.

Brock J, Caudill S, Silva M, Needham L, Hilborn E. 2002. Phthalate monoester levels in young children. Bull Environ Contam Toxicol 68:309–314.

Chapin RE, Robbins WA, Schieve LA, Sweeney AM, Tabacova SA, Tomashek KK. 2004. Off to a good start: the influence of pre- and periconceptual exposures, parental fertility, and nutrition on children's health. Environ Health Perspect 112:69–78.

Clark K, Cousins I, Mackay D. 2003. Assessment of critical exposure pathways. In: The Handbook of Environmental Chemistry, Vol. III, Part Q (Staples CA, ed). Berlin:Springer-Verlag, 22–262.

Colón I, Caro D, Bourdony CJ, Rosario O. 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ Health Perspect 108:895–900.

FDA. 2001. Safety Assessment of Di(2-ethylhexyl) Phthalate (DEHP) Released from PVC Medical Devices. Rockville, MD:Center for Devices and Radiological Health, U.S. Food and Drug Administration. Available: http://www.fda.gov/cdrh/ost/dehp-pvc.pdf [accessed 28 May 2004].

Gray L, Wolf C, Lambright C, Mann P, Price M, Cooper R, et al. 1999. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p'*-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane suphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol Ind Health 15:94–118.

Harris CA, Henttu P, Parker MG, Sumpter JP. 1997. The estrogenic activity of phthalate esters *in vitro*. Environ Health Perspect 105:802–811.

Jobling S, Reynolds T, White R, Parker, MG, Sumpter JP. 1995. A variety of environmentally persistent chemicals, including some phthalate plasticizers are weakly estrogenic. Environ Health Perspect 103:582–587.

Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. 2003. The OECD program to validate the rat uterotrophic bioassay. Phase 2: Coded single-dose studies. Environ Health Perspect 111:1550–1558.

Kessler W, Numtip W, Grote K, Csanady G, Chahoud I, Filser J. 2004. Body burden of di(2-ethylhexyl) phthalate (DEHP) and its primary metabolite mono(2-ethylhexyl) phthalate (MEHP) in pregnant and non-pregnant rats and marmosets. Toxicol Appl Pharmacol 195:142–153.

Kessler W, Phokha W, Csanady G, Filser J. 2001. No background concentrations of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in blood of rats. Arch Toxicol 75:62–64.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, et al. 2003. PPAR-alpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 33(6):655–780.

Kluwe W. 1982. Overview of the phthalate ester pharmacokinetics in mammalian species. Environ Health Perspect 45:3–10.

Li S-T, Lozano P, Gross D, Graham E. 2002. Hormone-containing hair product use in prepubertal children. Arch Pediatr Adolesc Med 156:85–86.

Lovekamp-Swan T, Davis BJ. 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ Health Perspect 111:139–145.

Lovekamp-Swan T, Jetten AM, Davis BJ. 2003. Dual activation of PPARa and PPARg by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. Mol Cell Endocrinol 201:133–141.

McKee RH, Butala JH, David RM, Gans G. 2004. NTP Center for the Evaluation of Risks to Human Reproduction reports on phthalates: addressing the data gaps. Reprod Toxicol 18:1–22.

Moore N. 2000. The oestrogenic potential of the phthalate esters. Reprod Toxicol 14:183–192.

Moore R, Rudy T, Lin T, Ko K, Peterson R. 2001. Abnormalities of sexual development in male rats with *in utero* and lactational exposure to the antiandrogenic plasticizer di(2-ethylhexyl) phthalate. Environ Health Perspect 109:229–237.

Mylchreest E, Cattley R, Foster P. 1998. Male reproductive tract malformations in rats following gestational and lactational exposure to di(n-butyl) phthalate: an antiandrogenic mechanism? Toxicol Sci 43:47–60.

Mylchreest E, Sar M, Cattley R, Foster P. 1999. Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. Toxicol Appl Pharmacol 156:81–95.

Parks L, Ostby J, Lambright C, Abbott B, Klinefelter G, Barlow N, et al. 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 58:339–349.

Peck C, Albro P. 1982. Toxic potential of the plasticizer di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. Environ Health Perspect 45:11–17.

Picard K, Lhuguenot J-C, Lavier-Canivenc M-C, Chagnon M-C. 2001. Estrogenic activity and metabolism of N-butyl benzyl phthalate in vitro: Identification of the active molecules. Toxicol Appl Pharmacol 72:108–111.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103(suppl 7):113–122.

Tiwary C. 1998. Premature sexual development in children following the use of estrogen- or placenta-containing hair products. Clin Pediatr 37:733–740.

Zacharewski T, Meek M, Clemons J, Wu Z, Fielden M, Matthews J. 1998. Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. Toxicol Sci 46:282–293.

Editor's Note: In accordance with journal policy, we attempted to contact Osvaldo Rosario, the corresponding author, to ask whether he wanted to respond to this letter, but our attempts have not been successful.

Correction

Karmaus et al. detected errors in their article "Backward Estimation of Exposure to Organochlorines using Repeated Measurements" [Environ Health Perspect 112:710–716 (2004)]. In Table 1, values in the equations for the proposed regression model were incorrect. The correct equations for Table 1 are as follows:

For estimation of 1970s values from 1980s values,

$$PCB\_70_{(estimate)} = PCB\_80 * 0.565 + (\varphi\_80 * -0.163) + (\omega * 0.106)$$

For estimation of 1980s values from 1990s values

$$PCB\_80_{(estimate)} = 10^{[-0.193 + (\log 10\ PCB\_90 * 0.781) + (\varphi\_90 * 0.049) + (\eta\_90 * -0.145)]}$$

Also, in Figure 3, there should not be a measurement for the year 2010.

The authors appologize for the errors.