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Christine Todd Whitman, Administrator
Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Ave. NW
Washington, DC 20460

Re: Comments on the revised DuPont HPV test plan for 5-carbon mononitriles (posted March 20, 2003)

Dear Administrator Whitman:

The following are comments on the revised test plan for the category five-carbon alkene mononitriles (5-C mononitriles), prepared by DuPont Co. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal, and environmental protection organizations have a combined membership of more than ten million Americans.

First, we commend DuPont for its decision to include within a single category four compounds and their stereoisomers. This means that data are only needed for the category as a whole (test plan, p. 1). The four compounds are all unsubstituted five-carbon monoalkene mononitriles: 2-methyl-3-butenenitrile (CAS no. 16529-56-9), cis- and trans-2-pentenenitrile, and a mixture of stereoisomers (CAS nos. 13284-42-9, 25899-50-7 and 26294-98-4, respectively), cis- and trans-3-pentenentriol, and a mixture of stereoisomers (CAS nos. 4735-87-4, 16545-78-1 and 16529-66-1, respectively), and 4-pentenenitrile (CAS no. 592-51-8). As a result of the similarity in molecular structure, all four compounds have identical molecular weights, and similar aqueous solubilities, boiling points, vapor pressures, specific gravities and partition coefficients (test plan, p. 3). They also have similar bioaccumulation potentials and fugacities (test plan, p. 4).

DuPont recognizes that, with respect to most of the endpoints covered by the HPV program, relevant data of acceptable quality are already available for the 5-C mononitriles. Numerous previous vertebrate studies are referred to in the test plan, including eight with 2-methyl-3-butenenitrile (summaries, pp. 22-29), twenty with 2-pentenenitrile (summaries, pp. 43-67), nine with 3-pentenenitrile (summaries, pp. 81-89), and ten with 4-pentenenitrile (summaries, pp. 100-107). In addition, we have found reports of two other toxicity studies that were carried out in rats. One study was on the acute toxicity of 2-pentenenitrile administered by injection (Tanii 1990). A second was on the effects on hepatic metabolism of 3-day repeat-dose oral administration of 2-pentenenitrile (Ozierenski 1993).

Although a large amount of animal toxicity data is available, DuPont maintains that no adequate SIDS data have been identified for reproductive or developmental toxicity (test plan, pp. 6-7). The test plan therefore states that a combined repeat-dose/reproductive/developmental toxicity test will be carried out, in accordance with OECD Guideline 422, in order to generate the required data (p. 9). This test would kill at least 675 animals. Our first concern with the testing proposal is that a developmental toxicity study on 2-pentenenitrile has already been carried out in rats (summaries, pp. 65-67; Saillenfait 2000). DuPont states that this study is of low reliability "because an inappropriate method or study design was

used" (summaries, p. 67). No attempt is made to justify this statement, but it may be based on the fact that the study involved only a single dose (oral, 125 mg/kg, on gestation day 10). DuPont's criticism of Saillenfait's study would carry some weight had the results been negative, but the results were clearly positive, with 94% of embryos showing malformations. Furthermore, a study on explanted embryos showed fetal toxicity at doses of 5.0 mmol/L and above, with 28% dead and 72% malformed at 10.0 mmol/L (Saillenfait 2000).

All nitriles that have been investigated have been found to be teratogenic in rodents. For example, in the above study, all seven other nitriles tested (acetonitrile, propionitrile, n-butyronitrile, acrylonitrile, methacrylonitrile, allylnitrile and 2-chloroacrylonitrile) were teratogenic (Saillenfait 2000). As a second example, eight aliphatic mononitriles (not 5-C mononitriles) showed teratogenicity and fetotoxicity in rats at inhalation doses above 25-200 ppm, and in the cases of several compounds the fetal effects occurred even when there was no maternal toxicity (Saillenfait 1993). As a third example, the intraperitoneal injection of two aliphatic nitriles (acrylonitrile and propionitrile) to hamsters gave rise to severe teratogenicity (Willhite 1981). Teratogenicity has also been demonstrated in a number of other studies (Doherty 1983, Johannsen 1986).

For the following two reasons, it is probable that nitrile teratogenicity is due to cyanide, one of the products of nitrile metabolism (Davis 1981, Farooqui 1992): (i) nitrile-caused malformations (of the allantois, trunk and caudal extremities) are characteristic of those in embryos exposed to cyanide (Saillenfait 2000, Doherty 1982); and (ii) maternal administration of sodium thiosulfate, a sulfur donor that enhances conversion of cyanide to the less toxic thiocyanate (Volini 1981), provides protection at all except the highest doses (Willhite 1981), as it does with other cyanogens (Willhite 1982) and cyanide itself (Doherty 1982). The mammalian metabolism of 5-C nitriles is probably by the mixed-function oxidase system, similarly to that of other nitriles (Davis 1981). Furthermore, the amount of cyanide released is probably greater than that from many other nitriles, as cyanide release tends to increase with nitrile chain length (Davis 1981). Therefore, it is difficult to see any reason, either experimental or theoretical, why teratogenicity should not be expected with 5-C nitriles. This was acknowledged in an earlier submission for the HPV program, for 2-amino-2,3-dimethylbutanenitrile, submitted by Cytec Industries, Inc., and the following statement specifically included 2-pentenenitrile: "Based on the data available, it can reasonably be assumed that all nitriles would have the potential to produce similar adverse effects of embryolethality, fetotoxicity and teratogenicity in laboratory animals" (Cytec 2002, p. 27).

For the above reasons, we consider that 5-C mononitriles are highly likely to show teratogenicity in rodents. The occurrence of rodent teratogenicity does not offer overwhelming evidence for developmental toxicity in humans, when one takes into consideration the marked differences in cyanide metabolism and teratogenicity between rats and pigs (Tewe 1981), and even between rodent species (Doherty 1982, Farooqui 1992), as well as the differences in nitrile toxicity between birds, mammals and amphibians (Davis 1981). However, it does point to the fact that further rodent studies are not appropriate.

Instead, two approaches should be followed: (i) investigation of human toxicity, by means of exposure assessments and epidemiology studies; and (ii) reduction of human exposure. These approaches are discussed below. In advocating these approaches, we must stress that our goals are to prevent both the needless killing of animals and the birth of malformed human infants.

Apparently very little investigation of U.S. occupational exposure to 5-C mononitriles has been conducted. The test plan states that air monitoring has been carried out on three 5-C mononitriles (p. 10), but, rather oddly, it provides no results of this monitoring. These data should be provided. By searching the databases, we have been unable to identify any other exposure studies on these compounds, or even data on how many people are exposed to them. There is therefore a pressing need for exposure and epidemiology studies. If U.S. human exposure to 5-C mononitriles is too low for such studies, one possible alternative would be to carry them out in South Asia, as populations there are routinely exposed to high concentrations of 4-pentenenitrile, which is formed in the traditional techniques used for the manufacture of rapeseed oil (Dietz 1991, Porter 1991, Kloss 1994, Brown 1996, Agnihotri 1999).

With respect to reduction rather than investigation of exposure, the test plan includes the following statement:

> Adequate safety equipment, such as safety showers, eyewash fountains, and washing facilities should be provided in the event of an occupational exposure. Individuals handling mononitriles should avoid contact with eyes, skin and clothing, thoroughly wash any exposed area of the skin after handling, and avoid breathing any dust. (pp. 9-10)

However, the test plan does not state whether these procedures are actually put into practice or enforced, and the general vagueness of the wording suggests that protection from these compounds may not be pursued with the utmost vigor. We must therefore stress that every effort must be taken to reduce exposure, especially to women of childbearing age.

Given the information presented above, and the fact that understanding and reducing exposure to humans is more important, and much more effective, than obtaining additional toxicity data on the reproductive and developmental effects of 5-C mononitriles in rodents, we ask that DuPont employ 'thoughtful toxicology and reconsider its plan to kill 675 animals. As stated in the October 1999 amongst the EPA, industry, and animal protection organizations, section 8, "As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be *useful or relevant*."

Thank you for your attention to these comments. I can be reached at 757-622-7382, extension 1304, or via e-mail at JessicaS@PETA.org and I look forward to your positive response.

Sincerely,

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