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MC 28221T
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Dockett ID No. EPA-HQ-OW-2010-0192

Dear Colleagues,

This letter constitutes public comment on EPA's proposed changes to analysis and sampling test procedures in wastewater regulations, pursuant to 40 CFR Parts 136, 260, 423, 430, and 435[EPA-HQ-OW-2010-0192; FRL-9228-6]*Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures.*

Disclaimer

The following comments are those of the author, who serves as an Environmental Health Scientist at the Center for Occupational and Environmental Health, University of California, Berkeley, and as the Associate Director of the UC Berkeley Center for Green Chemistry. These comments do not necessarily reflect the views of the Regents of the University of California, the University of California, Berkeley, the UC Berkeley Center for Occupational and Environmental Health, or the UC Berkeley Center for Green Chemistry.

Abstract

This letter urges EPA to discontinue its reliance on the use of n-hexane (85% minimum purity, 99.0% minimum saturated C6 isomer, residue < 1mg/L.) as part of the test method for determining the concentration of oil and grease in waste water, as described in *Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures.* The letter urges EPA to encourage the use of safer alternatives, particularly those that do not require the use of extraction solvents, which is consistent with EPA's stated interests in advancing green chemistry solutions.

The letter presents three key points:

1) n-Hexane is a well-documented occupational health hazard;
2) Health effects from exposure to n-hexane can occur at exposure levels several orders of magnitude below the federal Occupational Safety and Health Administration's Permissible Exposure Limit of 1,800 mg/m^3 (500 ppm);
3) Reducing the commercial circulation of n-hexane by allowing methods that use solvent-free technologies would help mitigate worker exposures to n-hexane throughout its life cycle; this approach is consistent with EPA's commitment to the principles of green chemistry.

Background

Before the use of Freon[supreg] was banned under the Montreal Protocol, EPA defined oil and grease as Freon[supreg]-extractable material. Following Montreal, EPA conducted side-by-side studies of several extracting solvents on a variety of samples to determine how the values compared to Freon[supreg]-extractable material values and settled on the use of a method that relies on n-hexane (hexane extractible material, or HEM).

EPA's use of n-hexane in response to Montreal is similar to the unintended consequences for workers that resulted in the vehicle repair industry following California EPA's phase-out of perchloroethylene (perc) from use as a degreasing agent in the vehicle repair industry. Cal/EPA took this action after finding that perc was contaminating used-oil that was collected from automotive repair shops throughout the state. Because used oil is used as bunker-C fuel in ships and trains, perc contamination was leading to emissions of dioxins from engines burning bunker-C fuel. Perc was also identified as a water contaminant by publicly owned treatment works (POTWs).

Cal/EPA's action, though appropriate for environmental health reasons, resulted in the widespread adoption of hexane-based cleaning products in the California vehicle repair industry. As a consequence, in 2000 the State Health Department began to identify auto mechanics with n-hexane-induced peripheral neuropathy, a disease process attributable to n-hexane exposure and characterized by a progressive loss of sensory and motor function in the limbs, leading to disability and loss of employment (CDC, 2001).[1]

In 2001, the Occupational Health Branch issued a statewide Health Hazard Advisory to warn operators in the vehicle repair industry of the hazards associated with the use of about 35 products available on the market that contained hexane (HESIS).[2] This action resulted in a reformulation by the industry to heptane and other less-toxic hydrocarbons.

In the present case, U.S. EPA describes six oil and grease methods and proposes that only three methods that use n-hexane are acceptable. EPA believes that the solvent-defined definition of oil and grease measurements precludes use of any other extraction solvent or extraction technique.

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[1] Harrison R, et al. n-Hexane-Related Peripheral Neuropathy Among Automotive Technicians - California, 1999-2000. *Morbidity and Mortality Weekly Review* 2001; 50(45):1011-3. US Centers for Disease Control and Prevention. See http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5045a3.htm (accessed December 15, 2010)

[2] California Department of Health Services, Occupational Health Branch, Hazard Evaluation System and Information Service (HESIS). 2001. *Health Hazard Advisory: n-Hexane Use in Vehicle Repair*. See http://www.cdph.ca.gov/programs/hesis/Documents/nhexane.pdf (accessed December 15, 2010).

EPA further believes that, without extensive side-by-side testing, permit writers, permitees, and data reviewers lack a basis for comparing the n-hexane-based measurements to values obtained with other extraction solvents or techniques. Furthermore, EPA "lacks information about whether permit writers or permitees would value having more ways to extract oil and grease samples, or about how much effort they or others would be willing to exert to determine if the alternate values were equal to HEM values or convertible to HEM values by a conversion factor."

Further, EPA proposes to specifically exclude two ASTM oil and grease methods, D7066-04 and D7575-10 because neither method uses n-hexane to determine oil and grease as hexane extractable material (HEM). D7575-10 is of particular interest because it is a solvent-free process.

"Instead of n-hexane, ASTM D7575-10 uses a different extracting process, an extracting membrane, followed by infrared measurement of the materials in the sample that can pass through the membrane. Several other steps in D7575-10 significantly differ from 1664 including: Use of 10-mL sample aliquot from sample bottle vs. entire contents of 1-L sample; homogenization of samples; and the challenge of pushing solid oil and grease samples through a membrane. The results of a multi-laboratory study (OSS 2009) that the developer conducted as part of ASTM's evaluation of D7575 are in the docket."

It appears that the largest barrier for EPA in moving away from n-hexane in this standard is the potential difficulties associated with adopting a new measurement technology, such as ASTM D7575-10, even when this technology represents a safer alternative to the use of n-hexane.

The information presented below elucidates the health effects associated with exposure to n-hexane. The intent is to motivate EPA to reconsider this aspect of the regulation and to allow the use of measurement technologies that do not rely on n-hexane.

1) n-Hexane is a well-documented health hazard, primarily for workers.

Public health concerns about n-hexane exposure have focused primarily on the potential for exposure to cause effects on the nervous system. However, further research suggests that n-hexane is also a reproductive toxicant for males: it is toxic to the testes in rats (Boekelheide 1987; Martino et al. 1987; Boekelheide 1988; Nylen et al. 1989; Richburg 1994) but not mice (Dunnick, et al. 1989). The same protein cross-linking that affects neurofilaments also alters the microtubules in the Sertoli cells and hence affects the germ cells (Richburg 1994; Li & Heindel 1998). The literature also shows that low- levels of exposure may cause abnormality in visual color discrimination as well as neuropsychological effects.

Peripheral neuropathy

The best-documented toxicity of n-hexane is a peripheral neuropathy that causes sensory and motor dysfunction in the distal limbs of exposed individuals (Wang, Chang et al. 1986; Pastore, Marhuenda et al. 1994; Spencer, Kim et al. 2002). Animal studies demonstrate that metabolism plays an important role in the neurotoxicity seen after n-hexane exposure. Researchers have shown that the n-hexane metabolite 2,5-hexanedione produced an identical peripheral neuropathy to that found after exposure to hexane (Schaumburg and Spencer 1976). In addition, the relationship between the time to onset of neurotoxicity and serum levels of 2,5-hexanedione strongly implicates this

metabolite as the mediator of n-hexane neurotoxicity (Krasavage, O'Donoghue et al. 1980). In human studies, worker monitoring has utilized urinary excretion of 2,5-hexanedione as an indicator of n-hexane exposure and potential toxicity (Ahonen and Schimberg 1988; Prieto, Marhuenda et al. 2003).

Neuropathy caused by n-hexane occurs primarily in two settings. The most intense exposure is seen in recreational drug users who habitually inhale the hydrocarbon for its euphoric central nervous system effects ("glue-sniffer's neuropathy"). A more chronic exposure is typically encountered among workers. Numerous industries have been implicated (Yamamura 1969; Herskowitz, Ishii et al. 1971; Sanagi, Seki et al. 1980; Mutti, Cavatorta et al. 1982; Wang, Chang et al. 1986; Huang, Shih et al. 1991; Chang, Yu et al. 1993; Takeuchi 1993; Herbert, Gerr et al. 1995). n-Hexane-induced peripheral neuropathy as experienced by workers tends to be a progressive, sensory-motor effect, characterized by a progressive muscle weakness and expanding areas of numbness in the limbs.

Reproductive Toxicity

The same hexanedione (2,5-HD) metabolite associated with neurotoxicity has been identified as a testicular toxicant (Chapin, Morgan et al. 1983; Boekelheide 1987). Since the metabolism of n-hexane to 2,5-HD is catalyzed by the cytochrome P450 enzyme system via sequential O-1 hydroxylation and oxidation in the liver (Couri and Milks 1982), both the nervous system and the testis are exposed to circulating levels of 2,5-HD metabolite. Although the testis effect has been shown in animals, it has not been investigated in humans.

2-5-Hexanedione (2,5-HD) targets the Sertoli cells in the testis (Chapin, Morgan et al. 1983). This testicular cell type serves as the "nurse" cell for the developing germ cells and is essential for spermatogenesis. It contains a well-developed cytoskeleton that performs transport and support functions not dissimilar to that seen in the axon of the nervous system. Tubulin is proposed to be the molecular target for the toxicity of 2,5-HD in the Sertoli cell (Boekelheide 1987; Boekelheide, Fleming et al. 2003). 2,5-HD combines with protein lysyl amines to form pyrroles, and these have been implicated in the injury seen in both the nervous system and the testis (DeCaprio, Olajos et al. 1982; Boekelheide 1988).

In studies using the rat as a model species, 2,5-HD was administered as a 1% solution in drinking water for a 3 to 5 week period (Boekelheide 1987; Boekelheide 1988). As early as 2 weeks after beginning the exposure, the assembly of testis tubulin into microtubules was altered. By 5 weeks of exposure, there was evidence of Sertoli cell damage and loss of germ cells. Eventually, no differentiating germ cells could be seen in the seminiferous tubules, and testicular atrophy was prolonged with little evidence of recovery.

There are many similarities between the neurotoxicity and the testicular toxicity of 2,5-hexanedione in the rat. Boekelheide (Boekelheide 1988) and Boekelheide and Eveleth (Boekelheide and Eveleth 1988) found that in the rat, testicular toxicity occurs in the absence of neurotoxicity after high level exposure for a short time. In contrast, when the exposure was chronic and low level, neurotoxicity occurred in the absence of testicular toxicity. Although there are many studies describing neurotoxicity of hexane in humans, no studies have examined potential damage to the human male reproductive system.

Visual impairment

Several organic solvents have been shown to induce color vision defects (Gobba and Cavalleri 2003). The most sensitive test for acquired color defects used in toxicological research is the Lanthony Desaturated D-15. This test has been adopted by the Agency for Toxic Substances and Disease Registry (ATSDR) because it was specifically designed to detect subtle acquired defects (Geller and Hudnell 1997). Norms have also been established and age effects described for North American populations (Roy, Podgor et al. 1991). In general, organic solvents produce primarily blue-yellow color vision defects but may generate mixed blue-yellow and red-green defects in more severe cases (Blain and Mergler 1986). Both defect types are detectable using the Lanthony test. Most previous research has involved mixed solvents (Dick, Semple et al. 2000; Semple, Dick et al. 2000; Gong, Kishi et al. 2003), however, a few studies specifically involving n-hexane have been published.

n-Hexane has been shown to produce color vision defects (Raitta, Seppalainen et al. 1978) in industrial workers. Primarily blue-yellow color defects were found. Chang (Chang 1990) showed recovery of n-hexane-induced polyneuropathy after several years of removal from exposure but color vision defects remained. Issever et al. (Issever, Malat et al. 2002) showed very large blue-yellow and red-green color vision error scores in 26 young workers with exposure to n-hexane in the leather industry (for approximately 7 years; no concentrations were given). Their subjects were specifically selected because of symptoms of polyneuropathy indicating fairly severe exposure, which may explain why they found significant red-green as well as blue-yellow errors.

Most studies did not present the concentrations at which these effects were observed. Raitta et al (1978) reported visual effects from intermittent high occupational exposures to hexane; for 8/15 subjects, the work-air concentration was below 500 ppm and for 7/15 subjects, the "normal" concentration was only 10-50 ppm, but when the machinery broke down, high exposures up to 3000 ppm occurred.

Neuropsychological Effects

There are a number of lines of evidence to suggest the possibility that n-hexane exposure can cause persistent neurobehavioral deficit. The literature on neurobehavioral effects of mixed organic solvent exposure, although complex and methodologically imperfect, indicates that industrial exposure to mixed organic solvents may negatively affect memory, attention, and psychomotor speed (Baker 1994)). Several reports emphasize n-hexane (Armstrong 1995; Tsai, Chen et al. 1997; Hageman, van der Hoek et al. 1999; Yu, Lee et al. 2004); all examine workers with either high level exposures, or examine chronic exposure to mixed organic solvents, sometimes including heavy metals.

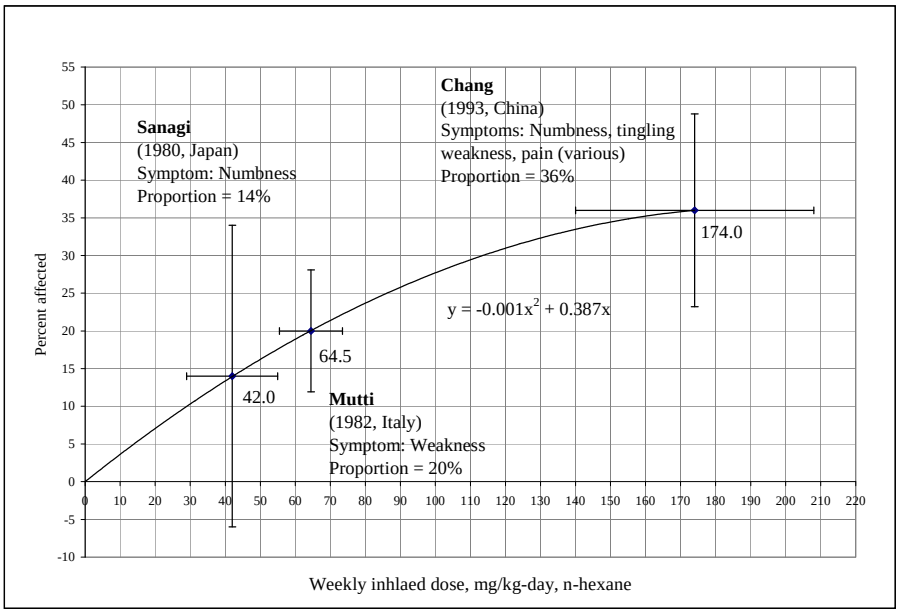
2) Health effects from exposure to n-hexane can occur at exposure levels several orders of magnitude below the federal Occupational Safety and Health Administration's Permissible Exposure Limit of 1,800 mg/m^3 (500 ppm);

There is great variability in the standards for n-hexane that have been recommended or applied, both for ambient exposures and in the workplace. US EPA recommends an ambient exposure level of 0.06 ppm, whereas ATSDR recommends 0.6 ppm and the California EPA 2 ppm. For the

workplace, the NIOSH Recommended Exposure Limit, the ACGIH Threshold Limit Value and the California OSHA Permissible Exposure Limit are 50 ppm, whereas the Federal OSHA PEL is 500 ppm. The occupational limits applied in other countries are similarly varied: the United Kingdom (20 ppm), Sweden (26 ppm), Hungary (28 ppm) and Japan (40 ppm).

Epidemiological studies have reported neurological health effects at n-hexane exposure concentrations under 100 ppm, suggesting there is little margin of safety. Sanagi and colleagues reported that 14 workers in a steel alloy manufacturing plant who were exposed to an 8-hour TWA n-hexane concentration of 58 ± 41 ppm showed a higher incidence of numbness and weakness in the extremities, along with significant differences in vibration sensation and subclinical neurological function, compared to 14 unexposed workers (Sanagi et al. 1980). Chang and colleagues reported 20 (36%) workers in an offset printing plant with symptoms of numbness, tingling, pain and weakness in the extremities from n-hexane exposure concentration ranging from 80 to 210 ppm (mean 132 ppm). An additional 26 (46%) workers in this study were identified with subclinical peripheral neurological deficits (Chang et al. 1993). Mutti and colleagues reported electrophysiological deficits and numbness, tingling and weakness in the extremities in 10-20% of 95 workers exposed to n-hexane at a median exposure concentration of 66 ppm (mean 90 ppm).(Mutti et al. 1982) (see Figure 1).

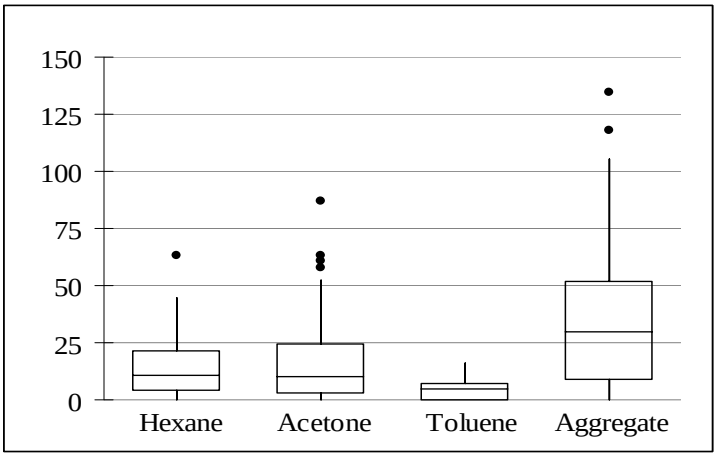
Figure 1. n-Hexane weekly inhaled dose response model, based on epidemiological data of Sanagi, Mutti and Chang. Exposure units are in mg/m^3. The federal OSHA PEL is 1,800 mg/m^3. The EPA RfC is 0.2 mg/m^3. The ATSDR MRL is 2.1 mg/m^3. The OEHAA Inhalation REL is 2.0 mg/m^3.



Our exposure studies in the vehicle repair industry suggest that the n-hexane TLV is not sufficiently protective. Using quantitative samples, all task-length n-hexane measurements and modeled full-shift n-hexane exposure estimates were well below the TLV, even for products formulated with 90% hexane (Wilson, 2007) (Figure 2). On reviewing the medical records and conducting interviews with individuals affected with n-hexane-induced peripheral neuropathy in this industry, we could find no

basis on which to conclude that their exposure to n-hexane had somehow exceeded the levels identified in our exposure assessment.

Figure 2. Concentration (mg/m^3) of total hexanes, acetone, toluene and total VOCs in 49 task-based area samples.



While the n-hexane TLV focuses on peripheral neurotoxic effects, the toxicological literature points to testicular damage in males as a second, and possibly more sensitive, health endpoint (see above).

3) Reducing the commercial circulation of n-hexane by allowing methods that use solvent-free technologies would help mitigate worker exposures to n-hexane throughout its life cycle; this approach is consistent with EPA's commitment to the principles of green chemistry.

Despite well-established evidence of its neurotoxicity, hexane (including n-hexane) continues to be a major industrial solvent in the U.S., and its use is increasing. In 2002, it passed for the first time the "one billion pound use-rate," as reported under the TSCA Inventory Update Rule. Hexane is widely used in products purchased by consumers and businesses, including paints, varnishes, cleaners, and degreasers. Given that health effects can occur at very low exposure levels, n-hexane poses a unique hazard, particularly in workplace settings.

EPA can and should take steps to reduce the commercial circulation of n-hexane, particularly when safer alternatives are available. Promoting green chemistry strategies is a "source-reduction" approach that prevents health and ecosystem damage "before it occurs." This damage can take place during the use of hexane-based products in their intended applications, but it also occurs throughout the chemical lifecyle, from processing at the refinery, to transportation, handling, storage, disposal. Green chemistry strategies seek to "design-out" the hazardous properties of chemical substances and chemical processes; they are a key element in US EPA's *Essential Principles for Reform of Chemicals Management Legislation:*

Principle No. 5. Green Chemistry Should Be Encouraged and Provisions Assuring Transparency and Public Access to Information Should Be Strengthened.

The design of safer and more sustainable chemicals, processes, and products should be encouraged and supported through research, education, recognition, and other means. The goal of these efforts should be to increase the design, manufacture, and use of lower risk, more energy efficient and sustainable chemical products and processes.

Conclusion

EPA should vigorously support the use of, and development of, measurement techniques to assess oil and grease in aqueous systems that no longer rely on the use of n-hexane. n-Hexane is a potent neurotoxic substance that produces a progressive peripheral neuropathy characterized by a gradual onset of numbness and weakness in the limbs. It also is associated with reproductive health effects (sterility) among males in animal studies, as well visual and neuropsychological effects. Most of the effects of n-hexane exposure, even when pronounced, are unlikely to be attributed (by workers or health care providers) to low-level exposure to n-hexane during the measurement of oil and grease in water, using an EPA-approved method. In our work in the vehicle repair industry, all 14 workers with n-hexane-induced peripheral neuropathy were misdiagnosed: in no case was an individual's symptoms associated with his occupational exposure to n-hexane. It is within reason to expect that workers conducting analytical tests using n-hexane will be unlikely to report their symptoms to their health care provider or to EPA. It is also within reason to expect that exposure levels could easily reach levels that would produce varying degrees of peripheral neurological disease, and perhaps reproductive dysfunction and visual impairment.

Preventing occupational exposures to n-hexane is best accomplished by promoting the use of safer, solvent-free technologies. This is consistent with the principles of green chemistry, as articulated by EPA's *Essential Principles* in 2010.

Please do not hesitate to contact me if you have questions regarding this or other aspects of n-hexane exposure or green chemistry solutions.

Respectfully submitted,

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