Diesel Exhaust

Materials for the December 4-5, 2008 Meeting of the California Environmental Contaminant Biomonitoring Program (CECBP) Scientific Guidance Panel (SGP)

Agenda Item: "Consideration of Potential Designated Chemicals"

Exposure or potential exposure to the public or specific subgroups:

High ambient exposures to diesel exhaust occur in ports, freeway intersections, and on school buses, with nearly all California residents exposed daily to significant levels (CARB 2005; SCAQMD 2000, 2008; Solomon et al. 2001). Diesel exhaust is a complex mixture of many chemicals including many polycyclic aromatic hydrocarbons (PAHs) and nitropyrenes. This document will review whether there might be a unique signature compound, or a few compounds, that could serve as diesel biomarkers.

PAHs, which are present in diesel exhaust, are already designated chemicals and are biomonitored by the U.S. Centers for Disease Control and Prevention (CDC). However, there are many sources of exposure to these compounds other than diesel. A highly specific diesel marker is clearly desirable given the health impacts of diesel exposure and the regulatory activities in California aimed at reducing exposure.

Known or suspected health effects:

Diesel exhaust is carcinogenic and is a major environmental pollutant. Environmental health agencies at the state, national and international level have identified diesel as a probable human carcinogen based on: (i) extensive experimental evidence of genotoxicity and of carcinogenicity in laboratory animals; (ii) data establishing the carcinogenicity of diesel exhaust constituents; and (iii) strongly suggestive evidence from human occupational studies (OEHHA, 1998; IARC 1989; WHO 1996; NTP 2000; U.S. EPA 2002; NIOSH 1998). Diesel exhaust is listed as known to the state to cause cancer under Proposition 65, and OEHHA considers diesel exhaust to be a human carcinogen (OEHHA 1998). Air pollutants associated with the use of diesel are an important public health concern in California because of high levels of these contaminants in certain urban and industrialized areas, especially around sea ports and near major highways. The California Air Resources Board (CARB 2005) found that "[o]verall, diesel engine emissions are responsible for a majority of California's estimated cancer risk attributable to air pollution." The South Coast Air Quality Management District's (SCAQMD 2008) "Multiple Air Toxics Exposure Study" (MATES III) found that 84 percent of the estimated cancer risk in the basin was due to diesel particulate matter, with an average basinwide risk of approximately one in one thousand. The highest risks were identified near ports and transportation corridors. Diesel is also a major contributor to premature death from cardiovascular and lung diseases and to asthma attacks and other respiratory effects, and it accounts for thousands of hospital admissions in the State annually (OEHHA 1998; CARB 2005).

Need to assess efficacy of public health actions

Diesel exhaust particles were formally identified as a Toxic Air Contaminant in California in 1998, and CARB has implemented numerous control strategies to reduce exposures in the State. In October 2000, CARB adopted a Diesel Risk Reduction Plan and has since been engaged in a

number of regulatory and other activities to address off-road, on-road and marine craft sources of diesel exposure. Details on these efforts can be found on CARB's web page: http://www.arb.ca.gov/diesel/mobile.htm. The continued findings of high risks to Californians, including large risk estimates in certain low-income communities located near sources of exposure, such as ports and highways, makes efficacy assessment of these actions particularly important.

Potential to biomonitor: Three approaches for biomonitoring for diesel are considered here. *1. Nitro-PAHs:* Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are a class of ubiquitous indoor and outdoor air contaminants that are derived from high-temperature combustion in the presence of nitrogen. Diesel particle emissions are enriched in 1-nitropyrene, produced during combustion. 1-Nitropyrene is not unique to diesel but is also present in other combustion sources. It is found in fireplace smoke, cooked meat products, airplane exhaust, and stack gases from coal fired power plants, and aluminum smelters (NTP 1996; IARC 1989). Both 1- and 2-nitropyrene exhibit sufficient evidence of carcinogenicity in laboratory animal studies and have been identified as carcinogens under California's Proposition 65 statute and by several national and international bodies (U.S. EPA 2002; IARC 1989; NTP 2000). Levels of 1-nitropyrene are in the ~pg/m^3 range – about a million times lower than the airborne particulate matter mass concentrations. Urban levels are higher than suburban levels in Japan (Murahashi and Hayakawa 1997). The highest reported levels (~130 pg/m^3) were measured during winter in urban/high traffic locations in Denmark (Feilberg et al. 2001).

2. Hydroxylated nitro-aromatic compounds: Air pollution and mechanistic studies suggest that the hydroxylated nitro-aromatic compounds may contain signature compounds that could serve as diesel markers for biomonitoring. During fuel combustion, large amounts of low molecular weight aromatic compounds (especially benzene, toluene and naphthalene) are formed. A fraction of the low molecular weight aromatic compounds is rapidly oxidized to hydroxylated aromatic compounds, most prominently to phenol, cresols, and naphthol. Hydroxylation of the aromatic rings in benzene, toluene and naphthalene activates the rings and facilitates nitration to produce nitro-phenols, nitro-cresols and nitro-naphthols. Chamber studies demonstrate that diesel engines typically emit two to three orders of magnitude more of these hydroxylated nitro-aromatic compounds than do gasoline-powered engines. It is likely that the hydroxylated nitro-aromatic compounds are eliminated in urine without being metabolized further. Thus, urinary nitro-phenols, nitro-cresols and nitro-naphthols could serve as useful diesel biomarkers. Further research is necessary to confirm this hypothesis (Marcia Nishioka, personal communication with Peter Flessel, CDPH).

3. Patterns based on PAH metabolites in tandem with other markers: As noted above, PAHs are already designated chemicals. PAHs are formed due to incomplete combustion of organic materials including fossil fuels, wood and tobacco. These compounds are also produced in cooking and are therefore plentiful in the diet. Urinary levels of hydroxy-PAHs have been widely used to characterize exposures to PAHs. In population studies, urinary hydroxy-PAHs have been shown to be significantly correlated with each other and with 1-hydroxypyrene, the biomarker considered as the gold standard for PAH exposure (Serdar et al. 2003, Waidyanatha et al. 2004). A number of PAHs are carcinogenic and it is of interest to examine exposure trends and group differences. Indeed, CDC, in the National Health and Nutrition Examination Survey

(NHANES), found large differences among study subjects in urinary levels of 1-hydroxypyrene and several other PAH metabolites. For example, even within a given age group, differences on the order of 5 to 10 are seen between the median and the 95th percentile, some of which may be due to diesel exposure. This should be explored further.

Because diet (e.g., grilled hamburgers) and smoking contribute to 1-hydroxypyrene and other PAH metabolite levels, these markers cannot be considered specific for diesel exhaust pollution. In order to improve the usefulness of these markers for diesel, measurements of urinary 1-hydroxypyrene and other PAH metabolites could be made in tandem with other markers of diesel exposure, such as metals in urine and total circulating serum immunoglobulin E (IgE).

One possible biomarker of diesel exposure to consider in combination with PAH metabolites is urinary levels of metals, particularly vanadium. Airborne vanadium is released by fossil fuel combustion, resulting in exposure by inhalation. However, vanadium is also found in other sources. Further work is required to determine the suitability of vanadium as a biomarker of diesel emission exposure in conjunction with PAH metabolites. (See "Vanadium" document for additional details.)

Another possible biomarker of diesel exposure to consider in tandem with PAH metabolites is total serum IgE. There is a growing body of literature suggesting a relationship between traffic-related air pollution and allergic disease. Animal studies have demonstrated that air pollution, particularly diesel exhaust, stimulates the production of IgE in serum. Exposure to traffic-related air pollution has been associated with increased IgE levels in children (Nordling et al. 2008) These findings suggest that IgE might be a useful biomarker for traffic-related air pollution. In addition, an increase in IgE in response to traffic-related air pollution is a biomarker of effect. However, the IgE response is validated as a generalized response to major contaminants in traffic pollution and is not a specific biomarker for diesel.

1-Hydroxypyrene (or other PAH metabolites), vanadium, and IgE are relatively easy and inexpensive to measure. Each biomarker reflects exposures to traffic-related air pollution as well as to other pollution sources. Using pattern recognition with 1-hydroxypyrene, metals and IgE data, it might be possible to discover patterns characteristic of various air pollution sources, including diesel. The deficiencies of this approach are that the relationship between diesel and these biomarker levels may be confounded by diet and smoking and they reflect exposure to traffic-related air pollution generally and not diesel specifically (Jane Gallagher, personal communication with P. Flessel). The three-marker approach would be interesting to explore for eventual possible use in biomonitoring for diesel exhaust exposure.

Past biomonitoring studies:
There are many studies of diesel exposure but few that attempt to measure diesel- specific metabolites in blood or urine. Metabolites of the nitro-PAH class, and 1-nitropyrene specifically, are not reported in NHANES 2003-2004. Recent published research identified 1-nitropyrene as a compound that could possibly serve as a potential biomarker for human exposure to diesel exhaust (Toriba et al. 2007). CDC is developing methods to identify urinary nitro-PAH metabolites (John Osterloh, personal communication with P. Flessel). In small pilot biomonitoring studies, 1-nitropyrene metabolite levels and 1-nitropyrene exposures in air were

highest in occupationally exposed groups in polluted cities and lowest in elderly subjects in Seattle (Chris Simpson, personal communication with P. Flessel). In an occupational study of taxi drivers in China, urinary levels of 1-nitropyrene metabolites increased with increasing 1-nitropyrene concentrations in ambient air (Toriba et al. 2008). In non-occupationally exposed Seattle subjects, there was no correlation between daily 1-nitropyrene exposures in air and first morning void 1-nitropyrene metabolite levels. Occupational studies have not demonstrated cross-shift changes in urine concentrations of 1-nitropyrene; these findings suggest that urinary 1-nitropyrene metabolite levels reflect chronic but not acute exposure to 1-nitropyrene (Simpson et al. 2008, C. Simpson, personal communication with P. Flessel).

Availability of analytical methods, availability of adequate biospecimens, and incremental analytical cost:

1. Nitro-PAHs: 1-nitropyrene. For 1-nitropyrene, a published analytical method is available (Toriba et al. 2007). Toriba et al. measured urinary metabolites of 1-nitropyrene in non-occupationally exposed people. Metabolites were extracted from (100 mL) urine and analyzed by LC-MS/MS (API 4000 Q Trap). The most abundant metabolites were hydroxy-N-acetyl-1-aminopyrenes (6- and 8-hydroxy-N-acetyl-1-aminopyrene) and hydroxy-1-nitropyrenes (6-and 8-hydroxynitropyrene), which were detected at levels of ~100 pmol/L of urine. The method was later applied in pilot occupational studies (Simpson et al. 2008, Toriba et al. 2008).

The disadvantages of using 1-nitropyrene are that the metabolites are present at very low levels, the analytical method is technically challenging and the test is currently very resource-intensive. Specific drawbacks to the method are discussed below:

- The method employs isotope dilution mass spectrometry, so that 1-nitropyrene metabolite levels can be detected at very low levels. The method could be run with instruments that CECBP has purchased (LC-MS/MS and GC-MS/MS). However, the isotopic standards required are not available commercially and therefore must be synthesized. Synthesis could be done in-house but would require a great deal of time and effort. It would be preferable to have the standards synthesized under contract. Once made, the standards would probably be stable (C. Simpson, personal communication with P. Flessel).

- The method requires a relatively large urine specimen (100 mL vs. a few mL typically used in biomonitoring studies.) The large sample volume could necessitate a change in specimen collection procedures.

- The method is specific for 1-nitropyrene metabolites. It could not easily be bundled to measure other nitro-PAH and PAH metabolites in a single analytical scheme.

At present, this methodological approach, while technically possible, would be challenging with current staff and instrumentation, and in the near term is probably not feasible. CECBP would need to direct most of the biomonitoring resources in the CDPH lab to measure 1-nitropyrene metabolites in urine on a routine, high through-put basis using the method of Toriba et al. (2007). However, further method developments are likely since diesel biomarker research is being actively pursued in multiple laboratories. CECBP will continue to monitor these developments.

2. *Hydroxylated aromatic compounds: Nitro-phenols, nitro-cresols, nitro-naphthols.* Air pollution studies demonstrate that hydroxylated aromatic compounds are found at much higher levels in diesel emissions compared to gasoline emissions. These compounds are likely to be present in diesel-exposed populations at much higher levels than metabolites of 1-nitropyrene. Also, analytical detection methods are available. Studies to confirm their presence in human specimens have not yet been published (M. Nishioka, personal communication with P. Flessel).

3. *Urinary 1-hydroxypyrene and supplementary metals and IgE markers:* As a biomarker for PAH exposure, 1-hydroxypyrene has several advantages. It is present in relatively large amounts and is easy and inexpensive to measure using well validated analytical methods (GC-MS/MS, LC-MS/MS). Urinary levels of many metals can be measured simultaneously using ICP-MS. Serum IgE is easy to test for and is routinely measured in many clinical laboratories.

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