studies with known C_H allotypes in families of various races are in progress.

The discovery of the Hv(1) allotypic determinant will be of considerable importance in (i) examining inheritance of immunoglobulin variable-region genes; (ii) evaluating current theories on the origins of antibody diversity (*18*); (iii) exploring the relationship and possible linkage between immunoglobulin variable-region genes and both constant-region and regulatory genes; (iv) elucidating possible associations between variable-region allotypes and certain immunodeficiency, autoimmune, and neoplastic diseases; and (v) examining the relationship between variable-region allotypes and the antigen receptor of T cells (*19*).

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Crankcase Oils: Are They a Major Mutagenic Burden in the Aquatic Environment?

Abstract. *Fractions from used crankcase oil enriched in polyaromatic hydrocarbons induced revertant colonies in* Salmonella typhimurium *strain TA 98 when activated by rat or trout liver extracts. The mutagenic activity was not due to benzopyrene or benzanthracene. Fractions from various crude and refined petroleums were nonmutagenic. Among various petroleum hydrocarbons entering inland and coastal waters, used crankcase oils may represent a major mutagenic burden.*

There has been considerable speculation concerning the mutagenic or carcinogenic (*1*) potential of polycyclic aromatic hydrocarbons (PAH) from petroleum spills in the aquatic environment (*2, 3*). Petroleum contains a complex of aromatic and heterocyclic compounds including the classical carcinogens benzopyrene (BP) and benzanthracene (BA) (*4*); besides these aromatic hydrocarbons, evidence has been presented for other carcinogenic compounds in oil (*5*). There is increasing speculation that some marine animal tumors may have a pollutant etiology (*6*), and the increase in PAH in the aquatic environment has generated concern about possible long-term adverse effects on the health of aquatic organisms as well as human consumers of fish.

Aromatic hydrocarbon hydroxylases (AHH) are involved in the bioactivation of aromatics to mutagens in mammalian systems, and these enzymes are now known to occur in most marine organisms (*7, 8*). Screening of chemicals for mutagenic activity is commonly performed by a procedure developed by Ames *et al.* (*9*). In the work reported here we used this method to test for the

Table 1. Mutagenicity of used crankcase oil fractions toward *S. typhimurium* strain TA 98. The results are means of two experiments, each performed in duplicate. Student's *t*-test was used for statistical evaluation. Background was taken to be the number of spontaneous revertants generated in (i) the absence of hydrocarbons or (ii) the absence of supernatant from the 9000*g* rat or trout liver fractions. Oil irradiation was carried out with a long-wave ultraviolet lamp on a DMSO extract at a distance of 25 cm for 2 hours. When DMSO extracts of crankcase oil were chromatographed on silica gel thin-layer plates (Macherey-Nagel, GF 254) and developed with benzene, at least six bands were noted. The active fraction, 5, corresponds to the band between R_F 0.35 and 0.6. Fractions were eluted with methylene chloride and evaporated to dryness, and the residues were dissolved in DMSO. Both BP and BA have R_F values > 0.65. Aromatic hydrocarbon hydroxylase activity was induced in trout on exposure to petroleum. Enzyme assays were carried out with supernatants from the 9000*g* liver fractions as previously described (*16*). Fractions were prepared from three or four pooled fish livers. To assay AHH, alkali-extractable fluorescence was measured at excitation wavelengths of 395 and 520 nm. These wavelengths are specific for the fluorescent 3-OH and 9-OH BP derivatives. Specific activity increased from 4 to 5 units in liver homogenates from control fish to 40 to 50 units in those from fish exposed to petroleum.

Test conditions	His+ revertants per plate
Used oil	45
Used oil plus 9000*g* rat liver fraction	415*
Irradiated oil plus 9000*g* rat liver fraction	535*
Fraction 5 plus 9000*g* rat liver fraction	600*
Fractions 1, 2, 3, 4, and 6 plus 9000*g* rat liver fraction	60
Benzopyrene (5 μg) plus 9000*g* rat liver fraction	240*
Used oil plus 9000*g* uninduced trout liver fraction	79
Used oil plus 9000*g* induced trout liver fraction	400*
Rat or trout 9000*g* supernatant	30

*Significantly above background, $P < .01$.

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mutagenicity of fractions of used crankcase oil activated by rat or trout liver extracts.

We first employed an Ames tester strain with fish extracts to see whether BP could be activated (*10, 11*). To check for BP activation, liver fractions were prepared from laboratory-reared rainbow trout (*Salmo gairdnerii*). Liver (2 to 3 g) was homogenized by hand in a 7-ml all-glass tissue grinder with 6 to 8 ml of buffer (0.05*M* tris-chloride and 0.25*M* sucrose, *p*H 7.5). Homogenates were centrifuged for 10 minutes at 9000*g* and the supernatants were frozen at −70°C. Preliminary experiments demonstrated that the number of revertant colonies produced was dependent on the level of AHH activity in the 9000*g* fractions. No attempt was made, however, to critically quantitate the number of revertant colonies produced with BP over a range of AHH activities. The results compared favorably with those obtained with 3-methylcholanthrene-induced rat microsomes.

Further work was carried out with PAH-enriched fractions from various crude oils and from used and unused crankcase oils activated with rat or fish extracts. Sprague-Dawley rats were used; liver fractions were prepared as for fish. Petroleum samples were made available by the American Petroleum Institute and included Venezuelan bunker, Kuwait crude, Louisiana crude, and a No. 2 fuel oil. The virgin crankcase oils included Texaco, Esso, Gulf, Irving, and Veedol brands. Used crankcase oil was obtained locally. Irradiated samples of the crude oils were also checked, since environmental weathering may produce toxic photooxidation products. Equal volumes of dimethyl sulfoxide (DMSO) and oil were mixed. After centrifugation, the DMSO layer was remixed and recentrifuged. This gave an effective emulsion-free extract. The DMSO served as both a good extractant for PAH and a vehicle for the introduction of chemicals to tester strains. A 100-μl portion of hydrocarbon extract was used in each study. Positive results were obtained only with used crankcase oils (Table 1), and it appears that compounds other than BP or BA are the major mutagenic sources. Mutagenesis was increased in extracts from fish exposed to petroleum for 3 to 4 days (*12*).

This work establishes that fish can produce mutagenic metabolites from PAH and that used crankcase oils, which are released into the terrestrial and aquatic environment in considerable quantities (*13*), may represent a considerable mutagenic threat. At present, it appears that control over crankcase oil disposal is minimal and difficult to regulate (*14*). Benzopyrene and benzanthracene, which are produced in automobile engines (*15*), appear not to be the major mutagenic components. Previous work in this laboratory (*16*) demonstrated that fish taken from environmental sites with a history of oil contamination had elevated AHH levels. In some mammalian systems there is a relationship between AHH activity and susceptibility to hydrocarbon-induced cancers (*17*). The public health hazard of crankcase oil, especially from occupational exposures and dirt road oiling, should also be reassessed.

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12. Rainbow trout (*Salmo gairdnerii*) were exposed to surface slicks of Venezuelan crude oil for 3 to 4 days. The slick was created by adding 10 ml of oil to a 16-liter aquarium (*8*).
13. There is evidence that crankcase oils may be a major source of waste petroleum in municipal waste water systems [J. T. Tanacredi, *J. Water Pollut. Control Fed.* **49**, 216 (1977)]. The amount of petroleum hydrocarbons from waste water effluents discharged into coastal waters in 1970 was approximately equal to the quantity reported from oil spills [J. W. Farrington and J. G. Quinn, *ibid.* **45**, 704 (1973)]. Of the petroleum hydrocarbons entering the ocean annually, 2.5 million tons may come from industrial wastes and municipal urban runoff compared to 2.1 million tons from marine transport (*3*).
14. In Canada, the Environmental Impact Control Directorate can account for only 20 percent of the used crankcase oil, which may be dumped, used in landfills or for road oiling, or burned.
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Psychophysical Functions for Perceived and Remembered Size

Abstract. *Separate groups of people estimated the sizes of perceived or of remembered objects. In three independent experiments, both sets of data were well fit by power functions, and the exponent was reliably smaller for remembered than for perceived size.*

Gustav Fechner (1801–1887) attempted to characterize the functional relationship between the psychological and the physical world and called this enterprise psychophysics. Although Fechner studied only the "lower mental activities" such as sensation, his conception of psychophysics included the "higher mental processes" as well, but he left for the future this exploration and the methods it might employ (*1*). Modern applications of signal detection theory (*2*), multidimensional scaling (*3, 4*), and reaction time (*5*) have revealed intriguing commonalities between perceptual and memorial processes. For example, some evidence (*3, 4*) suggests that a "second-order isomorphism" holds between objects and their (perceptual or memorial) internal representations, in that the structural relations among objects are mirrored by the corresponding functional relations among their internal representations. These perceptual-memorial similarities indicate that remembered stimuli may map onto physical values in the same way perceived stimuli do, but a sensitive comparison of perceptual and memorial psychophysical functions has not been reported (*6*).

We have used magnitude estimation, a direct scaling technique popularized by

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