Dioxins in diesel exhaust

SIR — Particulates (mainly soot) emitted from diesel engines can be controlled by inserting a ceramic 'regenerative trap' or filter into the exhaust pipe. The filter is cleaned (regenerated) when the carbonaceous deposit ignites and burns away. In real engines, ignition must be promoted either by heating (electrically or by injecting a slug of fuel) or by catalytically burning the deposit. This is achieved by adding an organic metal compound[1], for example copper, to the fuel. It decreases the quantity of soot formed in the engine and lowers the ignition temperature[2]. We show here, however, that particulate (soot) generation is ameliorated at the expense of increased dioxin emissions.

In the presence of a chlorine donor, dioxins/furans (PCCD/PCCF) can be formed by *de novo* synthesis in combustion gases as they cool through the range 250–400 °C (ref. 3). Diesel fuels contain small amounts of chlorine, which is known to form dioxins[4]. Certain metals can act as catalysts in these reformation reactions, copper being the most potent[5]. This raises the possibility that dioxin formation will be increased in a particulate trap system using copper additive in the fuel. To examine this possibility, we performed a series of tests using a small diesel engine driving an electrical generator, operated under normal conditions and when fitted with a regenerative soot trap.

We fitted a single-cylinder, 2.0-litre, indirect injection engine, rated at 3.5 kW nominal output with a cylindrical, commercial-grade cordierite monolith trap (Corning EX 66–100/17). The stock diesel fuel contained 0.9 p.p.m. chlorine as chloride. We prepared doped fuel by mixing 34.4 mg l^{-1} of copper into the stock fuel. We tested for emissions at various engine loads but constant speed using the stock fuel without the soot trap, and the copper-doped fuel with the soot trap.

The exhaust gases were withdrawn isokinetically through a probe, directed through the trap and into a US EPA method 23 sampling train, modified method 5 (ref. 6). The organic compounds were adsorbed onto specially purified XAD-2 resin which had been previously spiked with a range of stable isotope PCDD and PCDF surrogate standards. We weighed the particulates from the probe, filter, resin cartridge, trap and impinger. The contents from the sample train were extracted with organic solvents, purified and analysed by high-resolution gas chromatography and high-resolution electron impact mass spectrometry.

The figure (*a*) shows the particulate loadings of the exhaust gases, recorded as grams per litre of fuel used. The loadings with standard fuel increase by a factor of nearly three over the range of engine output. With copper present, the comparable particulate concentrations fall by 25 to 50%, with better results at higher outputs. This is consistent with the recorded behaviour of doped fuel[1].

The dioxin/furan results are reported on a toxic equivalent basis (I-TEQ) to allow for the different toxicities of the congeners (see *b* in the figure). Using the doped fuel, there is a significant increase in dioxin emissions, especially at low load. There are therefore conflicting environmental effects from the addition of copper to diesel fuels.

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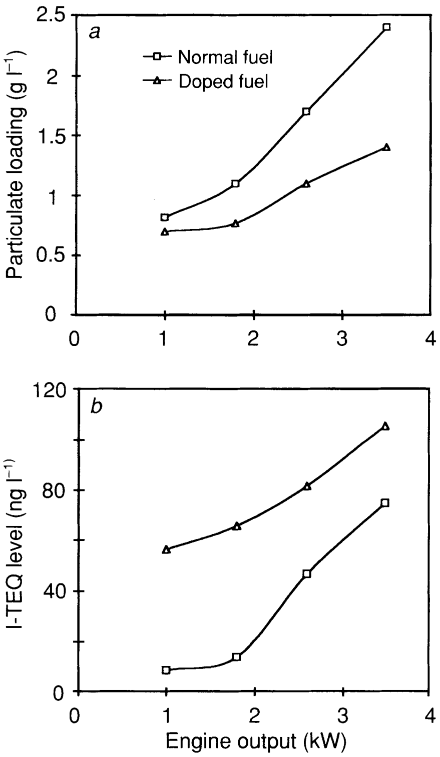


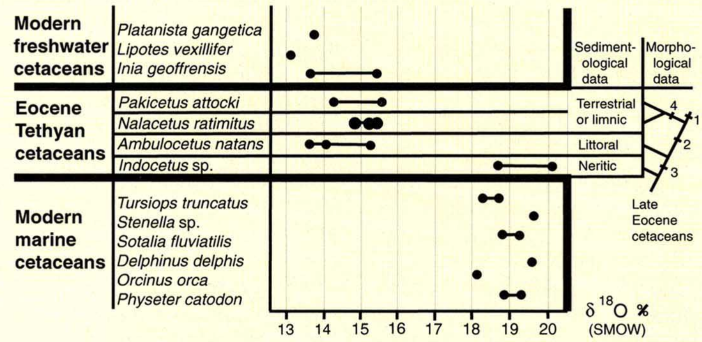
a, Particulate formation at different engine loads; *b*, PCDD/PCDF formation at different engine loads.

Evolution of cetacean osmoregulation

SIR — Cetaceans (whales, dolphins and porpoises) underwent dramatic changes during their evolutionary transformation from four-footed land animals to obligate swimmers. The morphological aspects of this transition have only recently been documented with fossils[1,2] and provide a striking example of adaptation to a new environment. However, equally remarkable changes must have occurred in aspects of cetacean biology that are not reflected in the gross morphology of fossils. Here, we use oxygen isotope evidence to document the early presence in cetaceans of one of the most extraordinary physiological adaptations to life in the sea: their ability to survive without fresh water. This adaptation made it possible for cetaceans to live offshore early in their evolution and allowed them to disperse across open oceans, sparking a worldwide radiation in the Eocene.

Most mammals cannot survive without a freshwater source, and marine mammals have adopted various osmoregulatory strategies to cope with the salt in their environment. In contrast to some other marine mammals[3], cetaceans[4,5] do ingest sea water, but it is unclear when the cetacean osmoregulatory system adapted to the excess salt load associated with ingesting sea water.

To address this problem, we determined the oxygen isotope composition of phosphate in teeth of early cetaceans. Biogenic phosphate, particularly tooth enamel, appears to be highly retentive of its original oxygen isotope ratio under moderate diagenetic conditions[6]. Mammalian teeth are mineralized in isotopic equilibrium with body water, the isotope composition of which is dominated by ingested water[7]. Thus, the oxygen isotope composition of bone and teeth predominantly reflects that of ingested water. As fresh waters are isotopically lighter than sea water, tooth phosphate of mammals ingesting fresh water will have lower $\delta^{18}O$ values than the teeth of mammals ingesting sea water[8]. Thus, the isotope composition of the teeth is a tracer of the broad categories (marine versus fresh) of water ingested. Factors in addition to the flux of ingested water may also influence oxygen isotope compositions[9,10]. We therefore tested whether ingested water dominates the isotope composition in cetaceans by analysing modern freshwater and marine species with a wide variety of diets and from a broad geographic range (see figure). The magnitude and direction of the



Oxygen isotope compositions of tooth phosphates of cetaceans. Isotope compositions are expressed relative to standard mean ocean water (SMOW) as $\delta^{18}O_P = [(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{SMOW} - 1]1{,}000$. Modern freshwater cetaceans have $\delta^{18}O$ values at least three per mil lower than those of marine cetaceans, consistent with previous findings[8,9]. As body temperature is nearly constant in mammals, the variation in isotope composition of mammalian teeth is a direct reflection of the variation in the isotope composition of the ingested water. The magnitude and direction of the differences of the fossil taxa are consistent with the difference among the modern cetaceans and with sedimentological evidence from the fossil sites in lower Kuldana and Harudi Formations. We analysed oxygen from the dental enamel or dentine (except in *Lipotes*, where only bone was available); each point represents a different individual. Our specimens of *Sotalia* and *Pontoporia* were marine. Phosphate was isolated as Ag_3PO_4, which was thermally decomposed to CO_2 in the presence of graphite[14]. Resultant CO_2 was analysed on gas-source mass spectrometers at the universities of Arizona and Michigan. Also summarized are inferred depositional environment and phylogenetic hypothesis for the Eocene taxa. Derived characters supporting the nodes are: 1, sigmoid process, rotated middle ear, pachyosteosclerotic tympanic and incus; 2, peribullar sinuses, enlarged mandibular foramen; 3, supraorbital plate; 4, P^4 with lingual bulge, lacking protocone.

differences in $\delta^{18}O$ values between freshwater and marine cetaceans reflect those of ambient waters, validating our method.

We analysed four of the oldest-known cetaceans. *Pakicetus*, *Nalacetus* and *Ambulocetus* are found in the early to middle Eocene Kuldana Formation of Pakistan. *Pakicetus* and *Nalacetus* are found only in shallow freshwater deposits[11] and probably spent considerable time on land. *Ambulocetus* occurs only in littoral beds[2]. *Indocetus* is known from the Middle Eocene Harudi Formation of western India and occurs only in neritic beds[12].

The difference in $\delta^{18}O$ values between *Pakicetus* and *Nalacetus* on the one hand and *Indocetus* on the other is similar in magnitude and direction to that between modern freshwater and marine cetaceans, suggesting that *Pakicetus* and *Nalacetus* ingested fresh water. This difference is consistent with habitat inferences from sedimentological data. Pakicetids, the geologically oldest whales, were clearly not marine. The $\delta^{18}O$ values of *Indocetus* are much higher than those of the lower Kuldana cetaceans and match those of modern marine cetaceans, suggesting that *Indocetus* ingested sea water only. *Indocetus* was thus totally independent of fresh water and the taxon was fully marine.

The $\delta^{18}O$ values of *Ambulocetus* are most similar to those of the other Kuldana cetaceans, implying that *Ambulocetus* ingested fresh water. This is surprising, as the taxon is found in unambiguously marine beds high in the Kuldana Formation[2]. Also, *Ambulocetus* has never been found in the freshwater deposits that abound in this part of the Kuldana Formation.

There are two possible explanations. *Ambulocetus*, although it lived in the littoral realm, may have sought out freshwater sources to drink because its osmoregulatory system was unable to handle the excess salt load of its environment. Alternatively, it may have lived in fresh water during the (early) part of its life when its teeth were mineralized, then migrated to the sea later on. This explanation lends credence to the idea that the life history of early cetaceans resembled that of modern pinnipeds[13].

Our analyses document the origin of seawater ingestion in whales. Our results imply that cetaceans did go through a stage, represented by *Ambulocetus*, in which they lived as adults in marine environments but depended, at least for part of their lives, on a freshwater source. However, by the middle Eocene, the osmoregulatory abilities of neritic cetaceans had adapted to increased salt loads, allowing them to leave the coast and disperse quickly across the oceans and colonize the globe. Dispersal ability presumably affected the pattern of cetacean macroevolutionary radiations, and may explain their distribution throughout the world's tropical regions only a few million years after their origin on the Tethyan shores of IndoPakistan.

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Redox regulation of cell signalling

SIR — We have been investigating the structural basis of the interaction between nitric oxide (NO) and the *ras* oncogene product p21 to gain insight into how redox signalling is achieved in cells. Our approach is one way to address the more general problem of finding a molecular target for redox-active environmental toxicants and free radicals. We report here that we have identified the site of molecular interaction between NO and $p21^{ras}$ that is responsible for the initiation of signal transduction.

In our earlier studies we found that a single *S*-nitrosylation event on full-length $p21^{ras}$ produced enhanced guanine nucleotide exchange[1]. Our present *in vitro* studies used $p21^{ras}$ lacking the carboxy-terminal 23 amino acids, as this form of the protein has identical biochemical activity to the wild-type enzyme[2]. To identify the exact site of *S*-nitrosylation, we cleaved $p21^{ras}$ with cyanogen bromide, which yielded three fragments, each containing one cysteine residue. Fragment 1, containing Cys 51, has a relative molecular mass (M_r) of 7,203; fragment 2, containing Cys 80, has M_r 4,540; and fragment 3, containing Cys 118, has M_r 6,225.