brief communications

Public health

Particulate emission from biomass combustion

ixing dried human sewage sludge with pulverized coal is being evaluated as a way to reduce carbon dioxide emissions from traditionally coal-fired power plants, as well as to overcome sewage-disposal problems. Here we investigate the effects of inhaling particles emitted from the combustion of this mixture and find that these cause significantly more lung damage in mice than do particles from coal alone, probably because of their zinc content. Our findings indicate that the use of dried municipal sewage sludge as a 'green' (CO₂-neutral) replacement fuel should be considered with caution.

Airborne particulate matter is associated with acute respiratory distress in humans¹. Suggested causes² of the lung damage caused by these particles include their composition — for example, they may contain soluble transition metals such as copper, iron, vanadium, nickel or zinc — their acidity, and their ultrafine size (some particles are less than 0.1 μ m in diameter). These properties are all features of airborne particulate matter resulting from the co-combustion of pulverized coal and biomass, including dried municipal sewage sludge (MSS).

Combustion of pulverized coal (from the University of Stuttgart) and of MSS/coal mixtures was performed in a semiindustrial-scale, 500-kW downfired pulverized-fuel combustor3. MSS had been processed (Swiss 'Combi' process) to produce 2-4-mm pellets, which were then pulverized and mixed (20% thermal, 50% mass load) with coal. Sampled particulate matter was resuspended and diluted⁴ to a sufficiently low concentration to allow the effects caused by different particle properties to be assessed on exposed mice. Mice were exposed for only 1 hour each day for 24 consecutive days to a low dose of 1,000 μ g m⁻³ and to a high dose of 3,000 µg m⁻³ (the atmospheric dose of particulate matter less than 10 µm in diameter currently allowed by the United States Environmental Protection Agency is 150 µg m⁻³ averaged over 24 hours).

Figure 1 indicates that co-combustion of MSS/coal ash produces airborne particulate matter that has a significant effect on lung permeability in mice, increasing with dosage, which is greater than the effect of either air on its own or a high dose of particles produced by burning only coal ash, which appears to be relatively benign. Cell counts in broncho-alveolar lavage fluid in our mice decreased after exposure to particulate matter from MSS/coal, compared to ambient air and coal ash alone, which again appears relatively benign (Fig. 1). (Details of measurement techniques and the use of



Figure 1 Effect of inhaled particulates on lung permeability (top) and broncho-alveolar lavage fluid cell counts (bottom). Thirty-two specific, pathogen-free mice (strain C57BL/6) were randomly divided into four groups (weight, 25.0 ± 4.3 g; n = 8), housed at 8 per cage under a 12-h light/12-h dark cycle at the Arizona Health Science Center animal facility (approved by the American Association for the Accreditation of Laboratory Animal Care, to ensure proper treatment of animals), and fed a standard chow diet and tap water. Besults were analysed 24-30 h after the final exposure to airborne particulate matter (see text). Cells were counted from the first 3 of 6 lavages (1 ml sterile isotonic saline) of the lungs. In the top bar graph, the asterisk symbol indicates statistical significance compared with exposure to particles from the coal/sewage ash of P < 0.0001; in the bottom graph, the asterisk and hash indicate statistical significance compared with exposure to coal/sewage ash particles of P < 0.0005 and P < 0.0001, respectively. Error bars indicate \pm s.d. Bars: 1, controls; 2, values corresponding to coal-ash exposure dose of ~3,000 $\mu g~m^{\text{-3}}$; 3 and 4, values for coal/sewage-ash exposures of ~1,000 $\mu g~m^{^{-3}}$ and ~3,000 $\mu g~m^{^{-3}}$, respectively.

anaesthetic and paralytic drugs to minimize discomfort and pain in the mice can be obtained from M.L.W.) We found no significant differences in other pulmonary functions (pulmonary compliance or resistance). Increased lung permeability enhances access by a toxin to the lung interstitium and pulmonary circulation, correlating with the extent of pathological lung injury.

We measured the distribution in particle sizes to which the mice were exposed by using a Berner low-pressure impactor, and recorded differences in mass and concentration of certain elements. Mass and size distributions for particles produced by coal/MSS ash mixtures and by coal ash on its own are quite similar, although the coal ash generates a slightly greater fraction of nanometresized particles. We conclude that the increased toxicity of the MSS/coal is not due to a difference in the size of the particles it produces on combustion.

Instead, the toxic effects seem to be associated with the presence of zinc in the particles. Combustion of MSS together with coal increases the concentration of zinc in the resulting airborne particulate matter in the respirable-size particles (diameters between 0.3 and 2.5 μ m) from 2 to 14 wt% and in ultrafine particles (diameter smaller than 0.1 μ m) from 4 to 11 wt%. MSS also produces

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10 wt% cobalt in the utrafines. Selenium, arsenic, lead and vanadium were present in similar amounts in particles from MSS/coal and from coal, whereas more iron was found in particles from coal burned alone. We therefore believe that zinc is the culprit metal that causes an increase in a measurable precursor for lung damage, a conclusion that is supported by other measurements⁵ of lung inflammation after intratracheal injection.

The replacement of coal by MSS in power stations is being considered as one option to help meet the requirements of the Kyoto Protocol and to replace landfill disposal of MSS. Our results, indicating that the toxicity to mice of inhaling particles from coal/MSS co-combustion is greater than from coal alone, suggest that the environmental advantages may be tempered by the risk to respiratory health.

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Palaeontology

An Early Cretaceous pellet

where have discovered a mass of fossil bones from four juvenile birds at Las Hoyas in Cuenca, Spain, which show signs of having been digested. To our knowledge, this rare finding of an Early Cretaceous fossil of an apparently regurgitated pellet provides the first evidence that Mesozoic birds were prey animals.

The new fossil assemblage contains four juvenile birds in an area smaller than 23 cm^2 (Fig. 1a,b). The two largest individuals (1 and 2; shown in dark and light grey, respectively, in Fig. 1) differ from one another in their tail morphology. In bird 2, the first seven caudal vertebrae are followed by a large pygostyle; in bird 1, the tail is composed of at least 12 free caudal vertebrae (Fig. 1c). The femur of bird 1 is 5–10% longer than that of bird 2, whereas the tar-

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Figure 1 Fossil pellet from the Lower Cretaceous of Las Hoyas (Cuenca, Spain). **a**, Bird assemblage from Las Hoyas (specimen LH 11386, Museo de las Ciencias de Castilla-La Mancha, Cuenca, found by a local collector, D. A. Diaz Romeral) showing feather impressions (arrow). **b**, Acid preparation of the specimen LH 11386. **c**, *Camera lucida* drawing of the bird assemblage from Las Hoyas. The most complete individual (bird 1; dark grey) shows preservation of almost the entire vertebral column in articulation and multiple portions of the appendicular skeleton in varying degrees of articulation. The second individual (bird 2; light grey) is identifiable from sections of its pelvis, sacral and caudal series, and hindlimbs. The remaining two individuals (birds 3 and 4; black) are significantly smaller than the first two. These specimens can be identified by only their hindlimb elements; at least three small metatarsi are present in the assemblage. Individuals 1 and 2 are of roughly the same size but significantly larger than individuals 3 and 4. For example, the tarsometatarsi of individuals 3 and 4 are about 60% and 50% shorter than those of individuals 1 and 2, respectively. **d**, Scanning electron micrograph (SEM) of specimen LH 11386 revealing surface damage (pitting and edge rounding) by digestion. Scale bar, 71.4 µm. **e**, SEM of a moderm avian bone digested by a jackal (courtesy of P. Andrews). Crocodiles produce very extreme digestion grades^{8,10}, higher than jackals and other canids⁵. Scale bar, 71.4 µm. **f**, SEM of an undigested modern juvenile bird bone (P. Andrews), showing absence of rounding and the characteristic porous and fibrous surface of juveniles. The specimen shown in **f** is larger than that in **d** or **e** and the magnification is reduced (scale bar, 667 µm) so that structural features can be compared.

sometatarsus of bird 1 is less than 85% the length of that of bird 2. The smaller individuals (birds 3 and 4) are comparable in size and have similar anatomical traits. The four individuals show a comparable degree of ossification. Morphological and size data suggest that at least three different species are present in the fossil.

This aggregation is peculiar to the Las Hoyas site^{1,2}, which has yielded more than 200 terrestrial vertebrate specimens that were usually isolated, fully articulated and complete. Results from oxygen and carbon isotope analysis of the Las Hoyas lacustrine limestones are in the range typical for sediments deposited in a still body of water³. Under these conditions, it would be unlikely that the four immature birds of this bone assemblage perished far apart and were then buried together in such a small area.

There are two more parsimonious explanations. The individuals could have been living in a nest that was washed into the lake, or their association could have resulted from predation as individuals and regurgitation by the predator combined into a pellet. The first of these two non-random explanations assumes nest parasitism, because the four individuals probably belong to three different bird species. Such a behaviour is unknown for any basal bird and cannot be confidently inferred from our knowledge of their closest relatives.

The idea that the fossil is a result of predation is supported by the corroded aspect of some articular ends, surface pitting and

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edge rounding of many of the bones (Fig. 1d), which look like digested bones from modern birds⁴ (Fig. 1e) and mammals⁵. These features differ from those expected from any other type of biological or geological erosion^{6,7}. The surface of bones from juvenile birds is also porous during periosteal formation, but this form of pitting is more fibrous and can be distinguished from that seen in digested bones (Fig. 1f).

Comparison with digested prey from extant predators indicates that the digestion of the Las Hoyas bone assemblage was extensive, although not as thorough as prey eaten by crocodiles⁸. The nearly intact anatomical connections of the bones contrast with the small size of bone fragments in dinosaur coprolites⁹. Impressions of complete feathers surrounding the assemblage of bones (Fig. 1a) resemble the feathers often seen enveloping modern pellets. These observations, together with the apparent absence of faecal ground mass, indicate that this multispecific aggregation of immature bird bones is a regurgitated pellet.

Identifying the predator responsible for this pellet is a problem. On the basis of the existing (and expected) fossil record from Las Hoyas, likely candidates could be large fish, early mammals, lizards, crocodiles (goniopholids), pterosaurs and dinosaurs (including birds), although some can be confidently excluded. Fishes and mammals regurgitate loose masses of bones, and the lizards and birds found at Las Hoyas are of comparable size to the pellet structure reported here. Goniopholids, close relatives of modern crocodiles, would probably have regurgitated more completely digested bones. The most likely predators to have produced the pellet are small, non-avian, theropod dinosaurs or pterosaurs that hunted different prey, swallowed them whole and then regurgitated the indigestible remains, much as owls do today.

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