Experimental study on the Effect of Ingested Lead Shot on Estuarine Crocodiles: Significance for Finniss River Field Studies

K.M. Hammerton

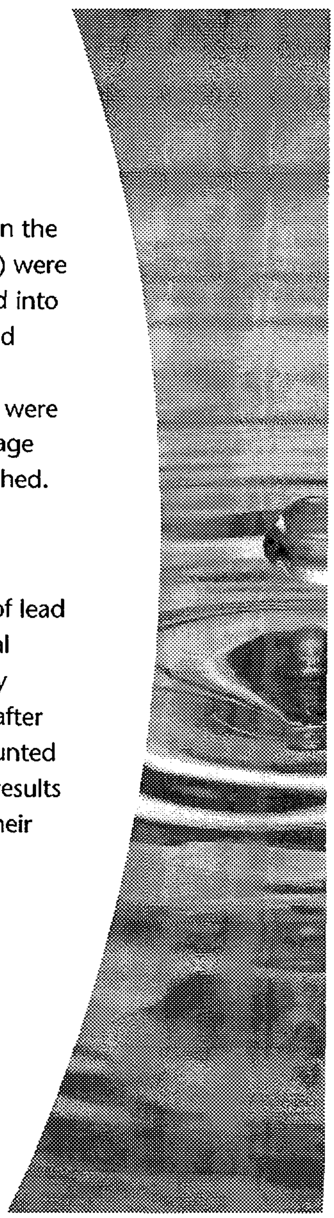
Environment Division, ANSTO, PMB 1 Menai, NSW 2234, Australia

Lead has long been recognised as a cumulative metabolic poison in humans, domestic animals and wildlife. Because of the many industrial activities that have brought about its widespread distribution, lead is ubiquitous in the environment. For example, uranium mining at the Rum Jungle site on the Finniss River, Northern Territory, resulted in contamination of river sediments with lead. Today, lead levels remain at about 250 mg kg^{-1} of wet river sediment within the immediate vicinity of the mine.

Another potential source of lead poisoning in wildlife is the use of lead gunshot for hunting. Mortality in wild waterfowl caused by the ingestion of spent lead shot has been recognised in North America and Europe for over a century. Secondary poisoning has been reported for several species of raptors that consumed prey containing lead shot. Previous studies at ANSTO showed that free-ranging estuarine crocodiles (*Crocodylus porosus*) sampled within areas in the Kakadu National Park, used for hunting by Aboriginals, contained elevated lead concentrations in their bones and flesh (Twining et al., 1999). As well, post-mortem examinations of crocodiles found dead in these areas found lead shot in their stomachs. It was proposed that crocodiles retain and dissolve lead shot in their stomachs followed by absorption into the blood and tissues that may result in lead poisoning. This proposal is summarised in **Figure 1**.

An experimental study was undertaken to assess the above hypothesis on the effects of lead in the environs of crocodiles. Six crocodiles (2 to 3 year old; length about 2 m; weight about 20 kg) were bred and housed at Crocodylus Park, Darwin. Lead shot (about 200 mg each) were packaged into kangaroo meat boluses and fed to the crocodiles. Three crocodiles were administered five lead shot (crocodiles 3T, 4T and 5T), one crocodile ten lead shot (crocodile 6T) and two control crocodiles (crocodiles 1C and 2C) were given pebbles to mimic the lead shot. Blood samples were taken at varying intervals over 20 weeks after lead shot ingestion. At 20 weeks a stomach lavage was carried out in order to recover retained lead shot after which the animals were radiographed. Further blood samples were taken over the next 22 weeks when a stomach lavage and radiography were repeated.

When the stomach lavage and radiography were performed at 20 weeks after the ingestion of lead shot, 18 of the 25 lead shot administered were accounted for. From 13 to 30% of the original weight of the lead shot had been dissolved in the stomach during the 20 weeks. Radiography revealed that two crocodiles (4T and 5T) had retained three and one lead shot, respectively, after the lavage. At 42 weeks after ingestion of lead shot, a stomach lavage and radiography accounted for two of these four lead shot. The weight loss for the lead shot recovered was 77%. These results confirmed the hypothesis that crocodiles have the ability to retain and dissolve lead shot in their stomachs. Throughout the experimental period the crocodiles remained in good physical condition and increased in their body weight and body length.



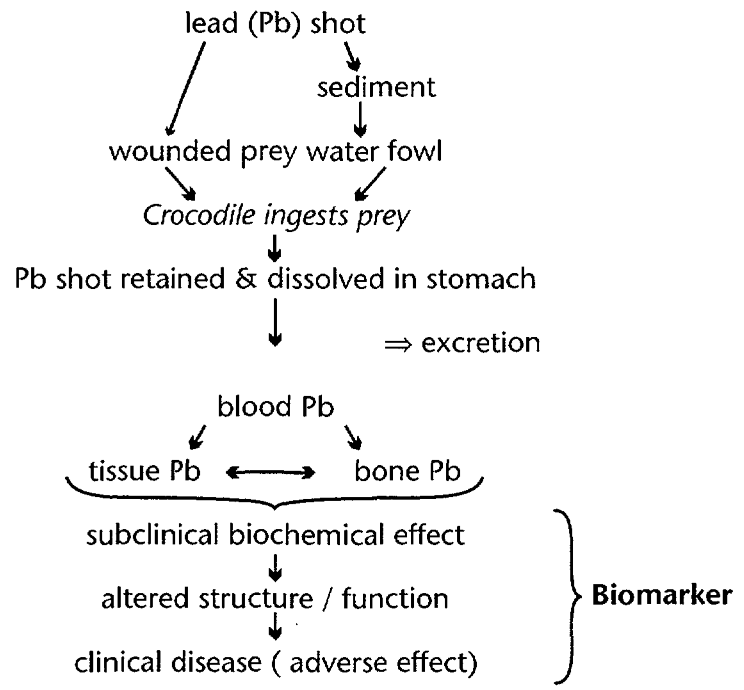


Figure 1. Flow chart of lead shot entering the crocodile from its environment.

Blood samples were diluted in 1% nitric acid and analysed for lead with inductively coupled plasma mass spectrometry. The background lead concentrations in the blood ranged from 10 to 30 µg dL^{-1} and remained within this range in the control crocodiles (1C and 2C). In the crocodiles dosed with lead shot, there was an order of magnitude increase in blood lead (BPb) during the first week after shot ingestion. The BPb in crocodiles dosed with five lead shot increased from pre-exposure levels to concentrations of 230, 300 and 320 µg dL^{-1} in crocodiles 3T, 4T and 5T, respectively, after which the BPb remained stable up to 20 weeks. In crocodile 6T, dosed with 10 lead shot, BPb increased to 550 µg dL^{-1} and remained stable. After the removal of the lead shot with the stomach lavage, BPb declined to pre-exposure levels over the subsequent 20 weeks. These results demonstrated that the dissolved lead from the lead shot was continually absorbed into the blood and a steady-state equilibrium between absorption, excretion and distribution to the tissues was maintained. Even though the steady-state levels of BPb were similar to those observed in birds after ingestion of lead shot, the rate of dissolution in the crocodilian stomach and the metabolism of lead were much slower than that obtained in birds.

Lead poisoning is a complex disorder and affects many organs in the body, including developing red blood cells, the kidneys and the nervous system. One of the most prominent effects is on the biosynthesis of haem, the prosthetic group in haemoglobin, cytochromes, catalases and peroxidases. The specific effects of lead intoxication arise mainly from the interaction of lead with the enzymatic processes in the haem biosynthetic pathway. One of these enzymes, δ-aminolevulinic acid dehydratase (ALAD), catalyses the condensation of two molecules of aminolevulinic acid to produce the pyrrole, porphobilinogen, the building block of the haem molecule. ALAD is a metalloenzyme requiring zinc for activity and is inhibited by lead displacing the essential zinc. This inhibition of ALAD by lead has been used as a specific biomarker for lead poisoning in fish, birds and mammals.

An assay system was developed for the measurement of ALAD activity in crocodilian blood. It was found that ALAD was inhibited by up to 90% during the first week after exposure of the crocodiles to lead shot. There was an inverse correlation between BPb and ALAD activity throughout the 42 week experimental period. BPb concentrations greater than 100 µg dL^{-1} produced significant inhibition of ALAD. The results indicated that ALAD inhibition could be used as a specific biomarker of lead toxicity in crocodiles.

The development of specific biomarkers of heavy metal contamination in river water and sediments will be very useful in determining the health of impacted ecosystems. Recently it has been demonstrated that the enzyme ALAD can be used as a bioindicator of lead pollution in riverine systems for vertebrates (fish; Nakagawa et al., 1998) and invertebrates (amphipods; Kutlu and Sumer, 1998). The biological specimens can be easily decontaminated of the lead in the sample and ALAD activity used as a specific biomarker of lead toxicity. The present studies on the effects of lead exposure in crocodiles and the development of suitable assay systems for biomarkers such as ALAD could be extended to evaluating the health of biota in pollution impacted riverine ecosystems.

References

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