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Determination of Zinc Phosphide and Related Heavy Metals in Biological Samples: A Case Report

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Zinc phosphide is utilized to control rats, mice, voles, ground squirrels, prairie canines, nutria, muskrats, wild rabbits and gophers. The aim of this study is to decide the kind of toxic substance uniquely zinc phosphide and to survey particular metal aggregation in different organs of the two bovines and measure the concentration of metals including Zn, Cd, Cu, Pb, which more often than not initiates a neurotic issue in the life forms. Analysis of tests by atomic absorption spectrometry (AAS) demonstrated high centralizations of Zn in liver, substance of stomach, lung and heart of both animals. Cd and Pb have abnormal states in a descending order (liver, substance of stomach, lung and heart). High level of zinc meddles with copper retention from the digestive system, bringing about copper inadequacy and in the long run in cardiovascular infections. In conclusion, the utilization of zinc phosphide may bring about critical harming cases, either unintentional in non-target species or here and there purposeful.

Keywords: Zinc phosphide, Toxicity.

INTRODUCTION

Zinc phosphide (Zn_3P_2) is a heavy, finely ground, crystalline dark powder that is practically insoluble in water and liquor. It was initially prepared in 1740 and was initially utilized as a rat's as a part of 1911 by the Italians. It was not utilized as a part of the U.S. as a rat's until 1939 when it was utilized as a part of a place of strychnine, which was hard to find due to rat control in Europe amid world war II [1,2]. Zn_3P_2 has been utilized as a part of USA to control jackrabbits, prairie canines, gophers, rats and voles [3].

It is utilized as a field rodent in Australia [4]. The prominence of zinc phosphide diminished amid the late 1940s and early 1950s, when sodium mono fluoroacetate (1080) and the counter coagulants initially showed up. As of late, as issues connected with sodium mono fluoroacetate (1080) and strychnine has been perceived, enthusiasm for zinc phosphide has again expanded [5,6]. Right now, zinc phosphide is perceived as the slowest acting of the usually utilized acute rat's [7].

Zinc phosphide is frequently suggested as the rodents of decision since it is genuinely particular for rodents and there is no genuine auxiliary poisoning, except possibly in dogs and cats. Most creatures that eat rodents are unaffected in light of the fact that the zinc phosphide does not collect in the rats' muscles or different tissues [8]. Infrequent accidental or more usually suicidal, exposure to zinc phosphide might be experienced. Ingestion, the main effectively dangerous course, has quite often been suicidal and the impacts acute [9].

Stephenson inspected 20 cases of acute zinc phosphide poisoning by ingestion (counting one treated by himself) in which the approximate dose was recorded. Ten cases were deadly and the doses ranged from 4.5 to 180 g; six cases had ingested 20 g or more. In the ten non-deadly cases, the doses ranged from 0.5 to 50 g with 7/10 ingesting under 20 g. For the situation treated by the author, clinical features included metabolic acidosis, hypocalcaemic tetany, methaemalbuminaemia and lessened blood coagulation (thrombotest 28 % of ordinary) [10,11].

Post-mortem included blood in all the serous cavities, pulmonary congestion and edema, hemorrhagic changes in the intestinal epithelium, centrilobular clog and necrosis and yellow staining of the liver and patchy necrosis of the rough convoluted tubules of the kidneys [12], the death rate of zinc phosphide harming is around 37-100 % [9].

The poisoning of Zn_3P_2 is essentially intervened by phosphine, which is shaped after hydrolysis of Zn_3P_2 on contact with stomach acids [13]. The interaction [$Zn_3P_2 + 6H_2O \rightarrow 2PH_3 + 3Zn(OH)_2$] is probably going to be acid-catalyzed and in this way to continue faster in the acidic environment of the mammalian stomach. Phosphine is promptly distributed all through the body to main organs, for example, the liver and kidneys [14].

Zn_3P_2 and phosphine harm the heart, liver, lungs and kidney and can bring about regurgitating, anorexia, abdominal pain, lethargy, hypotension, cardiac arrhythmias, circulatory collapse, pulmonary edema, seizures, renal damage, leukopenia and coma and demise in days to weeks [15].

Phosphorus causes behavioural reactions connected with inflammation of the stomach, covering and duodenum (*e.g.* a squatted/slouched pose) and the opportunity to death is more prominent than for cyanide and 1080, however shorter than for brodifacoum [16]. In more serious cases this may advance to cardiovascular crumple, pneumonic edema, cyanosis and respiratory fiasco.

Pericarditis, renal failure and hepatic harm, including jaundice, may evolve later. Indications might be late and death may happen up to one week in the wake of toxicity. Pathological discoveries incorporate greasy degeneration and necrosis of the liver and respiratory hyperaemia and oedema [17,18]. The onset of toxicity is quick after ingestion of Zn_3P_2 and as commonly happens during 15 min to 4 h after ingestion of a dangerous amount. Death from great dosage as commonly happens between 3 and 5 h [19]. The assessed lethal dosage is 40 mg/kg. Animals dosed orally with zinc component evolve pancreatitis, gastrointestinal and hepatic injuries and diffusenephrosis [20].

The deficiency Zn_3P_2 absorption over the skin is appropriate with the severe dermal LD_{50} in rabbits of 2000-5000 mg kg^{-1} [21]. To recognize zinc phosphide toxicity in an animal, phosphine gas must be distinguished. There have all the earmarks of being two groups in zinc phosphide, one which debases quickly and one which corrupts gradual in the wholesome tract. It is the gradually debasing part that must be recognized to affirm zinc phosphide toxicity. In the event that a suspicious case with zinc phosphide toxicity happens, the unopened carcass ought to be sent to the lab as fast as would be possible [22,23].

If the body has been scavenged and the stomach contents are exposed, they ought to be removed instantly and put in a hermetically sealed container and submitted to the lab [24,25]. At necropsy, the stomach contents ought to be tested and checked for the existence of an acetylene smell. The gathered stomach contents ought to be submitted for zinc phosphide testing at a 50 ppm detection level. Any rate at or over the 50 ppm level in the stomach contents are huge and demonstrate a zinc phosphide toxicity. Noteworthy qualities significant values are not available for other tissues at this time [26].

Heavy metal poisoning is an uncommon, but clinically noteworthy medical condition which can bring about different ailments, illnesses or even death if not perceived in time and treated suitably, which have the capacity to accumulate in tissues of body and their general potential to be poison even at comparatively minor levels of exposure [27].

Lead is a harmful metal that accumulates in the body and is related with subtle an insidious neurotoxic effects, which cause stomach suffering, unconsciousness, iron deficiency (anemia), infertility, nervous system disorders due to lead poisoning [28]. Acute cadmium ingestion in high dosage disturbs the gastric epithelium bringing about queasiness, vomiting, abdominal cramps and pains [29,30].

Zinc is fundamental for growth, spermatogenesis, skeletal advancement, skin defense, brain health and clinical improvement in atherosclerosis [31-33]. Exposure to unusually abnormal high levels of zinc can bring about different unfavourable health impacts.

Acute ingestion of elevated levels of zinc can bring about stomach spasms, uneasiness and vomiting [34]. Ingesting large amounts of zinc for several months may bring about sickness, harm the pancreas and diminishing levels of high-thickness lipoprotein cholesterol [35]. Interactions amongst Zn and different metals, for example, Cd, Ca, Cu and Fe happen during the process of its absorption and usage.

Cadmium take-up into the walls of the duodenum, jejunum and ileum are discouraged at high concentrations of zinc [34]. The aim of this study is to confirm the presence of zinc phosphide and to assess specific metal accumulation in various organs of the two cows and measure the concentrations of related metals including Zn, Cd, Cu, Pb which ordinarily induce pathological disorders in the organisms.

EXPERIMENTAL

The samples taken from liver, heart, lung and stomach contents of two poisoned cows collected during necropsy. The samples were packed in polyethylene bags and kept frozen at -20 °C. Before the analysis, samples were homogenized in a Teflon Ultra-Turrax Homogenizer [36].

All reagents must be of analytical grade *e.g.*, nitric acid (69 %), hydrofluoric acid (70 %), hydrochloric acid (70 %) and hydrogen peroxide (30 % v/v). Deionized water, resistivity 18.2 Mohm). Standard calibration solutions: Cd, Pb, Zn and Cu standard stock solutions conc. 1000 g/mL. Modifier for graphite furnace - atomic absorption spectrometry (GF-AAS), For Pb and Cd: Mix 1:1 of 0.2 % w/v Mg (NO_3) $2.6H_2O$ in 0.5 % v/v nitric acid and 0.2 % w/v $NH_4H_2PO_4$in 0.5 % v/v nitric acid.

Microwave Digestion-System, High Perfrmance from (ETHOS ONE), Atomic Absorption Spectrometer 240FS AA, from Agilent Technologies with (Graphite Furnace) GTA 120) æPSD 120 Programmable Sample Dispenser and carrier gas was argon.

Sample preparation

Zinc sulphide: The samples were acidified and placed in a round bottle flask which has stopper with an inlet tube which reaches the bottom of the flask and an exit tube connected to two traps one containing basic lead acetate solution for capturing H_2S and the second containing phosphine. The exit tube of the second trap is connected to a suction pump. The bottle is heated in a water bath. The current of air passing through the apparatus carries the gases evolved through the first trap which purifies the gases from H_2S and in the second trap phosphine combines with silver nitrate. The silver nitrate solution containing the silver phosphide precipitate are transferred into a beaker. Nitric acid is added and the mixture is boiled with the addition of hydrochloric acid and filtered to get rid of the silver chloride precipitate. The solution is evaporated to dryness. The residue is dissolved in nitric acid and ammonium molybdate solution is added, a yellow precipitate indicates the presence of phosphate.

Microwave digestion: Homogenized sub-samples (about 10 g) of each organ was digested by the method described by Hatem *et al.* [37]. Concentrations of Pb, Cd, Cu and Zn were determined by Agilent FAA Spectrometer FS 240 (Agilent Technologies, Inc., USA).

RESULTS AND DISCUSSION

Qualitative detection of zinc phosphide: The qualitative detection of zinc phosphide was indicated by the formation of a black precipitate of silver phosphate. It was proved by the digestion of samples of the dead body by concentrated sulfuric acid yields phosphine gas PH_3 as shown below:

$$3H_2SO_4 + Zn_3P_2 \longrightarrow 3ZnSO_4 + 2PH_3\uparrow$$

Phosphine, which evolved, reacts with the silver nitrate reagent and form silver phosphide (black precipitate)

$$3AgNO_3 + PH_3 \longrightarrow Ag_3P\downarrow + 3HNO_3$$

In the light of mentioned above, the amount of zinc is equivalent to the amount of phosphate (the toxic part) in zinc phosphide substance.

Metal phosphate are hydrolyzed to phosphine and the corresponding metal cation [38]. In rats, postpone that is not excreted in the expired air was oxidized and appeared in the urine, chiefly as hypophosphite and phosphate [39].

Meredith [39] also reported an unidentified metabolite, detectable by paper chromatography and distinct from pyro-phosphate and met-phosphate. The fact that (a) phosphine is incompletely oxidized and (b) the proportion of an administered dose that is eliminated as expired postpone increases with the dose suggests that the oxidative pathway is slow.

Zinc has been found in every tissue in the animal's body, but the highest concentrations occur in the prostate gland [40]. Concentration in the kidney, liver, heart and pancreas are also high [41]. The element tends to accumulate in the bones rather than the liver, which is the main storage organ of many of other trace elements. It is also involved in production, storage and secretion of hormones, in the immune system and electrolyte balance [40].

Zinc phosphide is also toxic to non-target mammals when ingested directly. Nearly 60 studies have been conducted on the toxicity of this Rodent's to wild animals. Secondary toxicity to mammalian predators (animals eating other animals that had been exposed to the compound) from zinc phosphide is rather low, primarily because the compound does not significantly accumulate in the muscles of target species.

Some of the toxic effects to of predators have been due to the ingestion of zinc phosphide that was in the digestive tract of the target organism. Studies on secondary organisms have focused on coyotes, fox, mink, weasels and birds of prey. Under field conditions, most of the toxic effects to non-target wildlife are due to direct exposures resulting from misuse or misapplication of this rodent's [42]. Zinc interacts with many chemicals to produce altered patterns of accumulation, metabolism and toxicity; some interactions are beneficial to the organism and others are not depending on the organism, its nutritional status and other variables [43].

Knowledge of these interactions is essential to the understanding of zinc toxic kinetics. The acute toxicity of zinc phosphide for large domestic animals was reported by Johnson and Fagerstone [8], where single doses were administered by capsule, stomach tube or mixed with the feed. Schlicker reported that 100 mg/kg body weight of zinc phosphide was fatal for starving dogs, but not for control. while exposed rabbits and a guinea pigs for 4 h per day to various concentrations of phosphine, the results show that, at dose 28 mg/m^3 (20 ppm), both rabbits and aguinea pigs died during or after the second exposure. At dose 14 mg/m^3 (10 ppm), rabbits survived 7-14 successive exposures, but at dose 12 mg/m^3 (8.3 ppm), only four or five exposures [44]. In another study in the series, two rabbits that had five 4-h exposures to phosphine at dose seven mg/m^3 were accidentally exposed to 20 mg/m^3 (14 ppm) on the sixth day. Both rabbits died during this exposure [45].

Data in the present study showed very high concentration of heavy metals, in liver, contents of stomach, lung and heart, which coincide with the qualitative examination, which proved the poisoning of the animals in this case (Figs. 1 and 2).

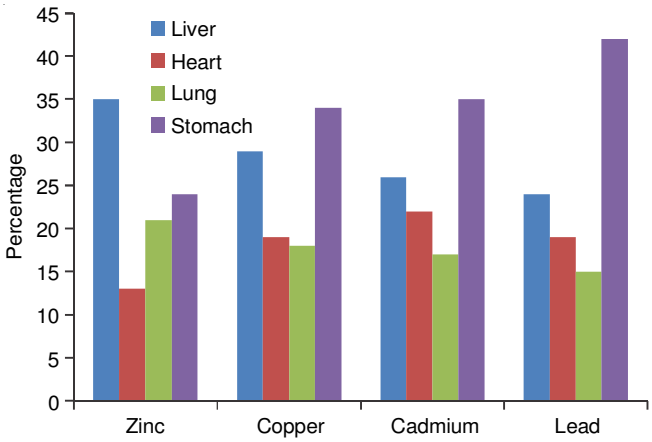

Fig. 1. Metals concentrations ratio in all organs of the animal (1)

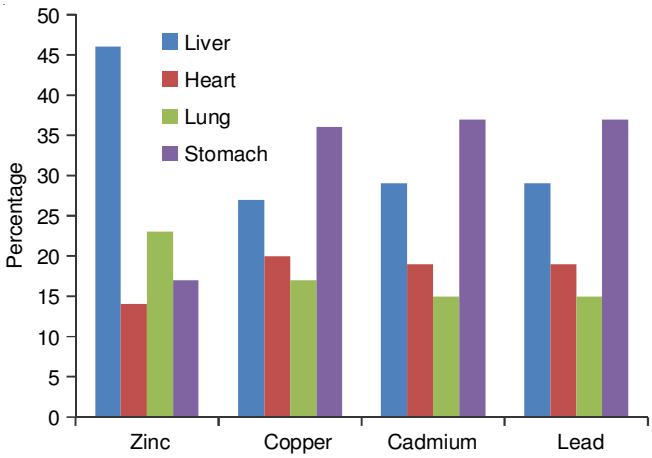

Fig. 2. Metals concentrations ratio in all organs of the animal (2)

The accumulation of zinc in different organs refers to the similar expected values of phosphorus in the dead animals. The data provide convincing evidence that the load of trace metals, in the body of the two animals is likely to reflect marked the specific test of the presence of poisoning of the animals (reaction with $AgNO_3$ to form a black precipitate) in Fig 1. Data showed that a higher accumulation in liver than hearts

and lungs, which affected on the metabolism and some enzymes, as shown in Figs. 1 and 2.

Dysfunction of hepatic fat metabolism was also observed. Loss of cell viability and cell membrane integrity accounted for the raised hepatic enzymes, the bronchiolitis effect, the cloudy swelling of renal tubular cells and the occasionally reported hemorrhagic lesions in the myocardium. The knowledge of trace metal specially zinc in different organs of the two animals, an important focal point to assess the acute impact of zinc phosphide poison on these endangered organisms.

Cadmium and lead are non-essential nutrients that are of direct concern to human and livestock health and may accumulate in the body, particularly in the kidney, liver and to a lesser extent in the muscle. Only a limited number of instances have been reported where levels in cattle tissue exceeded maximum acceptable limits for human consumption [46], but the present work has shown that high levels accumulate in the body of animals in this case in a descending order (liver > contents of stomach > lung > heart), which suggest that this high concentration may be resulted from a contamination of food of the animals in this case by substances contain Cd and Pb. High levels of administering zinc limit copper uptake in humans and certain animals and provides protection against toxicosis produced by copper in pigs and sheep [47].

Excessive zinc in humans interferes with copper absorption from the intestine, resulting in copper deficiency and eventually in cardiovascular diseases; high zinc intakes also decrease iron bio-availability, leading to a reduction of erythrocyte lifespan by 67 %, Copper deficiency induced by excess dietary zinc is associated with lameness in horses, donkeys and mules [7,17].

The acute toxic effect of zinc have been observed in sheep consuming zinc as a result of environmental contamination such as diarrhea, proteinuria, intestinal and adrenal lesions and pancreatic acinar cell degeneration [48]. In laboratory studies, hepatic and gastrointestinal lesions and pancreatitis appeared in sheep treated with 33 mg Zn/kg/day (as zinc sulfate) for 13 days [48]. Pancreatitis, diffuse nephrosis, intestinal hemorrhages and anemia were observed in ferrets given 850 mg Zn/kg/day (as zinc oxide in the diet) for 9-13 days [49]. This dose level was lethal to one of three animals.

A dose level of 425 mg Zn/kg/day for 7-21 days also resulted in nephrosis, pancreatitis and anemia, as well as fatty infiltration of the liver. A dose level of 142 mg Zn/kg/day for up to six months had no adverse effects. Copper deficiency and hypochromic microcytic anemia in humans chronically exposed [15,50].

Conclusion

The use of zinc phosphide may result in significant poisoning cases, either accidental in non-target species or sometimes intentional. If proper care is taken, the rodenticide can be safely used and its impact on non-target species minimized.

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REFERENCES

1. G.S. Bumbrah, K. Krishan, T. Kanchan, M. Sharma and G.S. Sodhi, *Forensic Sci. Int.*, **214**, 1 (2012); https://doi.org/10.1016/j.forsciint.2011.06.018.
2. S.W. Casteel and E.M. Bailey, *Vet. Hum. Toxicol.*, **28**, 151 (1986).
3. C.A. Ramey, J.B. Bourassa and J.E. Brooks, *Int. Biodeter. Biodegrad.*, **45**, 223 (2000); https://doi.org/10.1016/S0964-8305(00)00068-8.
4. M. Smith, B. Dyer, A. Brodie, W. Hunt and L. Staples, in eds.: G.R. Singleton, L.A. Hinds, C.J. Krebs and D.M. Spratt, Overcoming Rat Infestation in Australian Sugarcane Crops Using an Integrated Approach that Includes New Rodenticide Technology, In: Rats, Mice and People: Rodent Biology and Management, ACIAR Monograph 96, Australian Centre for International Agricultural Research, Canberra, pp. 238–241 (2003).
5. T.W. Clarkson, in ed.: R. Krieger, Inorganic and Organometal Pesticides, In: Handbook of Pesticide Toxicology, Academic Press, San Diego, Calif, USA, pp. 1357-1428 (2001).
6. R.T. Meister, Farm Chemicals Handbook, Meister Publishing, Willoughby, Ohio, USA (2001).
7. S.A. Briggs, Basic Guide to Pesticides: Their Characteristics and Hazards, Hemisphere Publishing Corp., WA (1992).
8. G.D. Johnson, andK.A. Fagerstone, Primary and Secondary Hazards of Zinc Phosphide to Nontarget Wildlife: A Review of the Literature, Denver Wildlife Research Center, USDA/APHIS, Denver, CO (1992).
9. O. Sogut, Z. Baysal and B. Ozdemir, *J. Emerg. Med.*, **40**, e117 (2011); https://doi.org/10.1016/j.jemermed.2009.05.039.
10. J.B.P. Stephenson, *Arch. Environ. Health*, **15**, 83 (1967); https://doi.org/10.1080/00039896.1967.10664880.
11. E. Charles, R. James, B. Helen and F. Alastair, *N. Z. J. Ecol.*, **37**, 1 (2013).
12. J.V. Murphy, *J. Am. Med. Inform. Assoc.*, **212**, 2119 (1970); https://doi.org/10.1001/jama.1970.03170250073021.
13. R.T. Sterner, *Pestic. Sci.*, **55**, 553 (1999); https://doi.org/10.1002/(SICI)1096-9063(199905)55:5<553::AID-PS936>3.0.CO;2-V.
14. K.G. Nikolaishvili and N.O. Melashvili, *Soobshcheniya Akademii Nauk Gruzinskoi SSR*, **144**, 429 (1992).
15. R.B. Mack, *North Carolina Med. J*., 50, 17 (1989).
16. C.E. O'Connor, K.E. Littin, L.M. Milne, A.T. Airey, R. Webster, D.G. Arthur, C.T. Eason and N.G. Gregory, *N. Z. Vet. J.*, **55**, 109 (2007); https://doi.org/10.1080/00480169.2007.36751.
17. R. Saxena, R.S. Bedwal and R.S. Mathur, *Trace Elem. Med.*, **6**, 119 (1989).
18. T.W. Clarkson, in eds.: W.J. Hayes and E.R. Laws, Inorganic and Organometallic Pesticides, In: Handbook of Pesticide Toxicology, Classes of Pesticides, Academic Press, NY, vol. 2 (1991).
19. K. Parton, A.N. Bruère and J.P. Chambers, Veterinary Clinical Toxicology, VetLearn 249, Palmerston North, Massey University, edn 3, pp. 143-155 (2006).
20. D.J. Ecobichon, in eds.: M.O. Amdur, J. Doull and C.D. Klassen, Toxic Effects of Pesticides, In: Casarett and Doull's Toxicology: The Basic Science of Pesticides, Pergamon Press, NY edn 4, (1991).
21. EPA, U.S. Reregistration Eligibility Decision (RED) Zinc Phosphide, Prevention, Pesticides and Toxic Substances, EPA 738-R-98-006, Washington, DC, U.S. Environmental Protection Agency (1998).
22. E.F. Straube, N.H. Schuster and A.J. Sinclair, *J. Comp. Pathol.*, **90**, 355 (1980); https://doi.org/10.1016/0021-9975(80)90003-1.
23. S.R. Ostrowski, E.W. Gunter and T.D. Matte, *Vet. Hum. Toxicol.*, **32**, 53 (1990).
24. Toxnet, Hazardous Substance Database, Zinc Phosphide, National Library of Medicine, Hazardous Substance Data Base, Publication date 9/93 (1992).
25. F.G. Guale, E.L. Stair, B.W. Johnson, W.C. Edwards and J.C. Haliburton, *Vet. Hum. Toxicol*., **36**, 517 (1994).
26. C.G. Elinder, in eds.: L. Friberg, G.F. Nordberg and V.B. Vouk, Zinc, In: Handbook on the Toxicology of Metals, Specific Metals, Elsevier, New York, edn 2, vol. II (1986).

27. J. Buchancova, M. Knizkova, D. Hyllova, M. Vrlik, D. Mesko, G. Klimentova and E. Galikova, *Cent. Eur. J. Public Health*, **2**, 82 (1994).
28. A. Khalid, I. Bukhari, M. Riaz, G. Rehman, Q. Ain, T. Bokhari, N. Rasool, M. Zubair and S. Munir, *Int. J. Biol. Pharm. Allied Sci.*, **2**, 1003 (2013).
29. H. Hu, in ed.: M. McCally, Human Health and Heavy Metals Exposure. In: Life Support, The Environment and Human Health, MIT press, pp. 1-13 (2002).
30. ATSDR, Agency for Toxic Substances and Disease Registry, Toxicological Profile for cadmium. U.S. Department of Health and Human Services, Public Health Service (1999).
31. R. Baker, *Environ. Int.*, **16**, 231 (1990); https://doi.org/10.1016/0160-4120(90)90117-O.
32. J. Drebler, K. Schulz, M. Klemm, R. Schüttig, A. Beuthin and D. Felscher, *Arch. Toxicol*., **76**, 449 (2002); https://doi.org/10.1007/s00204-002-0357-3.
33. E.J. Underwood, Trace Elements in Human and Animal Nutrition, Academic Press, New York, edn 4, pp. 1-421 (1977).
34. ATSDR, Agency for Toxic Substances and Disease Registry, Toxicological Profile for Zinc, U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia (2005).
35. V.S. Leblond and A. Hontela, *Toxicol. Appl. Pharmacol.*, **157**, 16 (1999); https://doi.org/10.1006/taap.1999.8660.
36. W.B. David, Guide to the Homogenization of Biological Samples, Random Primers, Issue No. 7, pp. 1-14 (2008).
37. A.M.A. Hatem, N.J. Muhammad and S.M.A. Jawaher, *IOSR J. Environ. Sci. Toxicol. Food Technol.*, **6**, 82 (2013).
38. EPA U.S., Health Effects Assessment for Zinc (and Compounds), U.S Environmental Protection Agency, Office of Research and Development, Washington, DC. EPA/540/1-86-048 (1984).
39. C. Meredith, M.Sc. Thesis, Toxicological Studies on Zinc Phosphide, Department of Biochemistry, University of Birmingham, Birmingham, (1981).
40. V. Ye. Zaichick, T.V. Sviridova and S.V. Zaichick, *Int. Urol. Nephrol.*, **29**, 565 (1997); https://doi.org/10.1007/BF02552202.
41. Y.C. Yoo, S.K. Lee, J.Y. Yang, S.W. In, K.W. Kim, K.H. Chung, M.G. Chung and S.Y. Choung, *J. Health Sci.*, **48**, 186 (2002); https://doi.org/10.1248/jhs.48.186.
42. C.D. Klaassen, Casarett and Doull's Toxicology, The Basic Science of Poisons, McGraw-Hill, New York, edn 6, pp. 801 (2001).
43. P.S. Rainbow, F. Liu and W.X. Wang, *Aquat. Toxicol.*, **162**, 102 (2015); https://doi.org/10.1016/j.aquatox.2015.03.007.
44. S.A. Schlicker and D.H. Cox, *J. Nutr.*, **95**, 287 (1968).
45. Zinc, United States National Academy of Sciences, National Search Council, Subcommittee on Zinc, University Park Press, Baltimore, MD. pp. 471 (1979).
46. A. Andrews and G. Henrique, Heavy Metal Pollution and Agriculture, TSM454Public Information Bulletin (2006); http//www.epa.gov/healthef/zinc.html.
47. M.J. Werman and S.J. Bhathena, *J. Nutr. Biochem.*, **6**, 373 (1995); https://doi.org/10.1016/0955-2863(95)80005-W.
48. D.N. Fiske, *Am. J. Hematol.*, **46**, 147 (2015); https://doi.org/10.1002/ajh.2830460217.
49. G.J. Fosmire, *Am. J. Clin. Nutr.*, **51**, 225 (1990).
50. F.J. Farrell, *Am. J. Ind. Med.*, **12**, 331 (1987); https://doi.org/10.1002/ajim.4700120308.