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The harm of organic arsine feed additives and its effects on environment

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ABSTRACT

Arsenic is a kind of necessary trace elements of livestock and poultry nutrition, and plays an important role in the tissues and organs and metabolism, etc. With the rapid development of feed industry, the organic arsine preparations of feed additives have been widely applied in animal husbandry. But these organic arsine preparations are absorbed less in animal body, and large amount of them are directly discharged by the animal dung. The animal dung which contains a lot of arsenic is not harmlessly managed, and it has polluted the environment and endangered human health. The types, mechanism of action and applications of the organic arsine are briefly introduced in this paper. The toxicological effects, the harm to human and ecological environment, and the related treatment measures of the organic arsine are mainly reviewed. We put forward to forbid entirely the use of organic arsine preparations in feed industry as soon as possible.

Key words: Organic arsine; Feed additive; Arsenic toxicity; Harm; Environmental pollution

INTRODUCTION

Arsenic is the fourth cycle and the fifth main group element in the periodic table of elements, its abundance in crust is the twentieth, lower than that of Cu and Sn, but higher than that of Hg, Cd, Au, Ag and Se, and it is mostly formed by +5, +3, 0 and -3 valence [1]. Arsenic compounds are often used for pesticides, herbicides, pesticides and so on. In addition, the arsenic is the element which is found to prevent selenium chronic poisoning, and it is an antagonist for plumbum, mercury and iodine. Arsenic may promote the body metabolic function together with antibiotics and vitamin B₁₂. In 1909, the first organic arsine preparation aniline arsine was synthesized. From then on, the prologue of organic arsine application in animal production was opened. In 1946, 3-nitro-4-hydroxy benzyl arsonic acid was found to have a role in promoting growth of chicks. In the 1950s, the United States began to the application research of organic arsine in feed field. In China, arsanilic acid and 3-nitro-4-hydroxy phenyl arsonic acid were respectively approved for use as feed additives in 1993 and 1996. With the rapid development of feed industry, the applications of organic arsine as feed additives in animal husbandry are also increasingly wide. The good economic benefits have been obtained in the meantime, people are beginning to pay more attention to the residual organic arsine feed additives and their harmful effects on the environment [2, 3]. Arsenic toxicity and contamination has been paid more attention to by people all over the world.

THE ORGANIC ARSINE PREPARATIONS COMMONLY USED

4-Aminophenylarsonic acid

4-Aminophenylarsonic acid, its name of commodity called arsanilic acid (ASA), its molecular formula is $C_6H_8AsNO_3$, and it is a pentavalent organic arsine preparation which commonly used in animal production. Arsanilic acid is soluble in NaOH solution, slightly soluble in water and ethanol, and insoluble in chloroform and ether. It was mainly used in pharmaceutical manufacturing and used for the determination reagent of ammonium, cerium and zirconium ions. Arsanilic acid also has the function of killing bacteria, protozoa and spirochete, and can be used in the treatment of poultry bacterial infections.

3-Nitro-4-hydroxy benzyl arsonic acid

3-Nitro-4-hydroxy benzyl arsonic acid, its name of commodity called roxarsone (ROX), and its molecular formula is $C_6H_6AsNO_6$, which is white or pale yellow columnar crystal. It is odorless, and soluble in methanol, ethanol, acetic acid, acetone and alkali, and insoluble in ethyl acetate. Roxarsone is the most economical organic arsine preparation, and often used for feed additives of poultry and swine. It has the efficacies of promoting growth, dysentery treatment, deposition of pigment, antibacterial, anticoccidiosis and so on [4, 5]. Roxarsone can be used with a variety of antibiotics and growth promoting agents mixture, and can improve the utilization rate of feed and reduce the cost of breeding [6]. There are synergistic effects between roxarsone and some vitamins, but roxarsone has antagonism effects with a variety of trace elements [7]. At present, roxarsone is still allowed to use as feed additive in China, and its dosage is not more than 50 mg/kg in feed. However, it is difficult to degrade roxarsone in alimentary canal of animals, and about 80% of roxarsone is discharged into environment [8]. Under certain environmental conditions, roxarsone is transformed into more toxic inorganic arsenic.

THE ACTION MECHANISM AND APPLICATION OF ORGANIC ARSINE

The mechanism of organic arsine

The organic arsine in organisms often exists in the forms of pentavalent and trivalent arsenic, and both of them can be transformed into each other, so it not only plays a role of the reducing agent and oxidizing agent. Arsenic can be combined with the sulfhydryl oxidase in organisms, and make the loss of enzyme activity through the covalent bonds formed. Arsenic can enhance the assimilation and weaken the dissimilation, make hematopoiesis function enhancement, promote the excitement of the nervous system, enhance animal appetite, so as to improve nutrition, promote the growth of animals. But excessive arsenic can result in the cell metabolism disorders. The arsenic is mainly accumulated in the liver, kidney, spleen, bones, skin and hair after arsenic preparation is absorbed by the body.

The application of organic arsine in animal husbandry

The organic arsine preparations can strengthen the assimilation of animal body, enhance the hematopoietic function of bone marrow, stimulate metabolism, improve the digestibility of nutrients, and inhibit the growth and breeding of harmful microorganisms and parasites in the intestines. It also can increase the permeability of capillary, so the animal shows the appearance of red skin and bright hair. American FDA required that the diet dose of arsanilic acid was 50–100 mg/kg (chicken) and 250–400 mg/kg (grice), and the diet dose of roxarsone was 25–50 mg/kg (chicken) and 300–500 mg/kg (grice). In the last 20 years, the application ranges of organic arsine have been rapidly spread in China, and extend from pig and chicken to the other breeding field like livestock, poultry and aquatic products. The promoting growth range of organic arsine between appropriate dose and toxic dose is relatively narrow, so the dose added level of the organic arsine must be controlled strictly. In addition, because of high toxicity of the arsenic preparations and less dosage, it must be firstly dispersed with mountain flour or calcium hydrogen phosphate as a carrier before we use it, then can be added to the feed.

THE HARM OF ORGANIC ARSINE PREPARATIONS

Although the organic arsine has brought great economic benefits in the feed industry, but the harm is not be underestimated. In the United States, there were nearly 70% of the poultry feed containing roxarsone, and the vast majority of roxarsone were excreted with the feces [9, 10]. Most organic arsine preparations are discharged with the dung and urine in the form of methylation products, and its toxic effects is different with inorganic arsenic. The damage characteristics are the demyelination of peripheral nerve and optic nerve and the glial proliferation.

Toxicological effects of organic arsine

Any toxic effect of the toxic chemicals on the body is in molecular level, and then is acted on cells, tissues and organs, and the obvious toxic reaction is generated at last. Arsenic can kill the bacteria and parasites, and also can poison the host. The toxicity of organic arsine is not very high by itself, but it can be converted into inorganic arsenic in animal body. For most animals, the inorganic As^{3+} can only be methylation in the liver, and it can react with the proteins in the blood before it enters the liver. Thus it can destroy the structure of protein and induce the body's pathological changes. The arsenic which is not in vivo residual will be excreted with the droppings of poultry and livestock, and becomes the source of environmental pollution. The arsenic in soil exists in various forms, including trivalent monomethyl arsine (MMA) and dimethyl arsine (DMA), pentavalent monomethyl arsonic acid (MMAA) and trimethyl arsine oxide (TMAO) and so on [11]. Among them, the soluble arsenic and loosely bound arsenic in the animal body enters into the human body through the food chain, and endangers the human body health. Arsenic is a first-class carcinogenic chemical. Excessive intake of arsenic may lead to various cancers, also can result in teratogenic and mutagenic. Calculating by the LD₅₀ of arsenic compounds (mg/kg), the order of all kinds of arsenic toxicity from big to small is As^{3+} (14) > As^{5+} (20) > MMA (200–1800) > DMA (200–2600) [12]. The study found that the more the arsenic was associated with organic groups, the less its toxicity [13]. Arsenite can be

changed into arsenate with small toxicity under the photochemical effect.

Virus of organic arsine to animals

Most drug additives have a corresponding residue and enrichment, but the residual quantity is different, so does organic arsine preparations. When the pig poisoned, the first symptoms was that the weight increased slowly, the ataxia and paresis of hind limb. Further, the sick pigs like dog sit down or fall on the ground, gradually blind until death, has the symptom of dermatitis during the course of illness. The symptoms of chicken poisoning are in poor health, sink-head, ataxia, paralysis and blindness, besides the lower of the growth rate. In 1954, Abbott reported that the chicken decreased slightly growth before 3 weeks of age when the diet was added 1000 mg/kg arsanilic acid, and the chickens appeared similar to vitamin B_1 deficiency when the arsanilic acid was added more than 1500 mg/kg, and the chickens occurred a large number of death in 9-12 d when the arsanilic acid added was greater than 2000 mg/kg. Buck also made a similar study, found that the pig appeared poisoning symptoms after 3-6 weeks when adding 250 mg/kg of arsanilic acid, and the pig could appeared poisoning, muscle tremor, ataxia and even death when adding 106 mg/kg of roxarsone. Wang et al [14] studied the effects of roxarsone on the Na+-K+-ATPase activity in tissue (gill, kidney and liver) and DNA damage in kidney cell of crucian carp (Carassius auratus). The result showed that Na+-K+-ATPase activity of three tissues were significantly inhibited by roxarsone. The Na+-K+-ATPase activity decreased gradually as roxarsone doses and exposure time were raised, which showed dose-effect relationship and time-effect relationship. The extent of DNA damage increased gradually as roxarsone doses were raised, which showed dose-effect relationship. Roxarsone might have potential biochemical toxicity and genotoxicity to aquatic organisms. Wen et al [15] studied the effects of roxarsone on growth performance and blood indicators in pigs, the results showed that roxarsone can help to improve the growth performance and the capacity of oxygen transportation in blood. However, it may damage the livers and kidneys of animals.

Harm of organic arsine to human beings

"Environment Health Standard of Arsenic" published by the United Nations Environment Programme (UNEP) about safety assessment report of arsenic pointed out that long-term exposure to arsenic compounds had toxic side effects for many organ systems, and organic arsine toxicity showed the central nervous system disorders, and higher incidence of encephalopathy and optic atrophy. With the research progress on arsenic toxicology, the International Agency for Research on Cancer (IARC) released that arsenic and its compounds are the carcinogenic factor in 1980, they can cause lung cancer, bladder cancer, liver cancer, kidney cancer, skin cancer and so on [16]. People chronically exposed to arsenic environment are considered as the main cause of high incidence of skin cancer, lung cancer and so on, and the incubation period of these cancers is 30–50 years [17]. The symptoms of chronic arsenic poisoning are headache, insomnia, the persistent mucosa inflammation of nose, throat and other parts, muscle relaxation, loss of appetite and other symptoms, some people may happen the skin damage of melanin deposition, keratosis, alopecia, etc., and may develop into multiple neuritis, optic atrophy, chromosome abnormality and so on.

"Health Standards of Drinking Water" in China specified that the content of arsenic should not exceed 0.05 mg/L in the drinking water. Even arsenic in water reaches the toxic concentration (100 mg/L), because the arsenic will not change the color and transparency of the water, only water taste slightly changes, people often do not feel it, therefore this brings very big potential threat to people's health. At the same time, the determination of residual organic arsine is very complicated, and so far the content of the residual arsenic in the sales livestock products is not determined with high precision method, which brings great hidden danger to food safety. Generally, chronic arsenic poisoning has a regional [18], such as the waste water containing arsenic of the chemical fertilizer plants in Guizhou Province was discharged, resulting in large area of arsenic poisoning in the lower reaches. When arsenic in drinking water is 0.12 mg/L, the arsenic poisoning incidence is 1.43% after 10 years. The shortest incubation period of poisoning for the arsenic content of 0.6 mg/L area in drinking water is half a year and the incidence rate is 47.18% after 10 years. Henan and Inner Mongolia had found that the content of arsenic in drinking water exceeded the standard and the arsenic caused the cases of the endemic arsenic poisoning and the skin cancer induced. In 1990, the "beer arsenic poisoning" happened in British, more than 1000 people were killed, and more than 700 people were poisoning. The multiple black foot disease in Taiwan was caused by the high concentration of arsenic in groundwater. Arsenic pollution of groundwater in a livestock and poultry farm is very serious and has threatened drinking water security, and the pollution results from organic arsine feed additives.

The toxic dose of arsenic is 0.01-0.052 g, and the lethal dose is 0.06-0.2 g, the bearable dose of arsenic exposure is $0.3 \ \mu g/(kg \cdot d)$ [19]. Under the 90 mg/kg dose of arsanilic acid, the residual arsenic in the pork was 0.36 mg/kg when feeding the feed was stopped after 4 d. If a person eats 50 g of the meat in one day, 0.54 mg of arsenic will be eaten into in 30 d, and 6.48 mg of arsenic will be taken in one year, the sum is more than the lethal dose of 0.06 g arsenic in 10 years. The pharmacokinetic studies showed that the arsenic residues was maximum in liver, followed by kidney, and the minimum in the muscle after pigs were fed organic arsine [20], and the animal internal organs containing high arsenic such as liver and kidney were eaten by human beings. Wen et al [21] studied the changes of

the certain genotoxicities in workers occupationally exposed to arsenic, and the results showed that the content of inorganic arsenic and organic arsine in the workers' urine of a certain arsenic factory was all very high, and the crowd constantly absorbed inorganic arsenic and continuously entered organic arsine metabolic transformation. It has been confirmed that low concentrations of roxarsone and its degradation product As(III) can harm vascular system of the human body [22]. The factories of arsenic preparation production and the farms used long-term the organic arsine preparations, it is easy to make the incidence rate of skin cancer for the employees and the surrounding residents increase.

Effects of organic arsine on ecological environment

Organic arsine is easily absorbed by animals, but it is less deposited in the body, and its utilization rate is very low, the general is only 5% or so. The 95% of organic arsine is almost discharged by prototype with droppings [23]. The arsenic content in the droppings is 14–48 mg/kg when livestock and poultry are chronically fed the feed of organic arsine exceeded the standard [24]. If the processing and emission ways of the droppings are improper, organic arsine will seriously pollute environment [25, 26]. The migration and transformation of arsenic in nature as well as its risk to people's health have raised a widespread concern. There exist several ways of arsenic in soil: (1) the formation of an insoluble arsenate; (2) adsorption on the surface of soil clay and other insoluble metal salts; (3) dissolution in the soil solution. Arsenic by oneself or combine with other heavy metals, can reduce the quantity of soil microorganisms (bacteria, radiation bacteria, fungi and nematodes, etc.) and inhibit the activity of the enzyme. In addition, under the action of soil microorganism, the morphology, distribution and toxicity behavior of organic arsine will be changed, and arsenic can remain and accumulate in the soil through the physical and chemical reaction such as the adsorption and precipitation, ion exchange, complexation, redox reaction and so on.

The influence of different forms of arsenic on the environment and organisms is not the same. Some organic arsine compounds can be accumulated in soil, such as herbicides methanearsonic acid and dimethylarsenic acid, they easily enter from soil into plants body, and then access into the food chain. Dimethyl arsine in food can be dissolved and accumulated in fat, thus it has a cumulative function in the food chain. Arsenic affects the growth of plants by influencing the plant to absorb water and nutrients and damaging chlorophyll of plant, the most of arsenic absorbed by plants concentrates in the roots, stems and leaves. That the excessive arsenic in soil can harm plant growth and development has been confirmed by many researches [27], such as inhibiting the activity of root system, hindering plants to absorb and transport the nutrients like water, N, P, Mg, K and Ca and so on. The research result showed that if the arsenic content in soil was 20-30 times for the control area, the arsenic content in the stalks and leaves part of crop was 1.3–3.0 times, and the root part was as high as 4–5 times. If the arsenic content in soil increases 1 mg/kg, and the arsenic content in the sweet potato tuber increases 0.28 mg/kg. According to this ratio calculation, the arsenic content in the sweet potato of the area will exceed the national food hygiene standard within ten years. Aspergillus, bacteria and saprophytic fungi can make the organic arsine compounds reduce into the smaller molecules of inorganic arsenicals, and it is the main way for the metastasis and redistribution of arsenic from the soil into the environment. Garbarino et al [28] reported that if each chicken was given normal doses of roxarsone, and these chickens would discharge a total of 150 mg roxarsone into the surrounding environment in the growth period of 42 d, and the farm waste samples were detected in 30-50 mg/kg arsenic (total arsenic). If a farm feeds 10 million feather chickens every year, then more than 400 kg of arsenic will be discharged annually to the environment.

The breeding industry has caused a serious pollution of organic arsine. Yao et al [29] investigated the 61 samples of manures from the intensive poultry and livestock farms of Guangdong province, the results showed that the content of arsenic in chicken and pig manure reached 21.6 mg/kg and 89.3 mg/kg, respectively. The arsenic content of the water body around the fish ponds of pig farms using organic arsine generally exceeded the standard, and the arsenic content in the neighboring soil of the pig farm sewage outfall even reached 200-500 mg/kg. In 1997, Academician Ziyi Zhang pointed out, if a pig farm of ten thousand head pigs continuously used the feed containing arsenic, and they did not adopt corresponding measures to deal with the faeces, then according to the calculation of FDA allowed the use of arsenic preparation dosage, the pig farm would discharge 1000 kg arsenic into the surrounding environment in 5-8 years later, and the arsenic content in the soil could increase one times after 16 years, at the same time, the arsenic content in groundwater would be increased accordingly. According to report, about 110 million kilogram of arsenic was annually discharged into the water bodies through the ways of runoff and biological actions all over the world [30]. Meng et al [31] made the relevant investigation for the ecological environment features of the Zhujiang Jiushawei River, a branch of Pearl River, and the results indicated that the water quality of monitoring section proved worse than the standard of grade IV with the organic pollution, the worst sediment pollution was organic arsine. Zang et al [32] made the samples analysis for the heavy metal elements in the soil of the mineral storage yards of Tianjin Port Harbor, and the results showed that the arsenic was significantly higher than that of local background. Its potential ecological risk factor was between 160 and 320, and the area was highly risk degree, the pollution levels were more than the grade III of the soil pollution standard. The concentration of organic arsine in groundwater is low, but the concentration of organic arsine will be increased in some industrial pollution. Guo et al [33] systematically discussed the metabolic residue and discharge of organic arsine and the degradation law in chicken manure by aerobic composting test, and roxarsone could cause serious damage to the ecological environment. The morphological analysis of arsenic and the purification and restoration in water, soil and sediment have been carried out some research in China [34, 35], but the arsenic pollution of environment is still serious, this research needs to strengthen continually.

GOVERNANCE MEASURES OF ORGANIC ARSINE

The serious harm produced by the organic arsine preparations has attracted the attention of people, solving the negative effects on the ecological environment has been imperative. At present, the main determination methods of organic arsine include the combined use of gas chromatography and mass spectrometry, high performance liquid chromatography [36] and inductively coupled plasma mass spectrometry and so on. Under the condition of microwave heating, using methanol-water mixture as arsenic extraction solvent has been proved to be simple and effective method. A method has been developed for the determination of inorganic arsenic and organic arsine in the excrement samples of organic arsenical feed-taking pig by hydride generation-atomic fluorescence spectrometry combined with microwave assisted extraction [37], this method can quickly determine whether animals poisoning on organic arsine. For the removal of organic arsine, Yan et al [38] reported the biosorption of inorganic and organic arsenic from aqueous solution by acidithiobacillus ferrooxidans BY-3, and Rivera-Reyna et al [39] explored the photocatalytical removal of inorganic and organic arsenic species from aqueous solution using zinc oxide semiconductor. As(V) can be removed from water mainly using physical and chemical adsorption methods, but it is difficult to remove As(III) without charges. Thus, changing the oxidation-reduction potential of anaerobic sludge to transform As(III) into As(V) will be favorable to removing roxarsone through anaerobic digestion, and it can reduce and eliminate environmental pollution caused on the addition of organic arsenic to feed [40]. The research results of Langner et al [41] showed that the natural organic compounds could completely isolate and fix arsenic through the formation of covalent bonds between trivalent arsenic and organic sulfur components under aerobic conditions, thereby the arsenic mobility in water or wetland was reduced, and the pollution range was decreased. Kumpiene et al [42] used the FeO particles removal arsenic in the drinking water, and acquired good results, the advantages of high efficiency and low cost made the method possess good application prospect. Chen et al [43] studied the adsorption of roxarsone and arsanilic acid on the surface of iron and aluminum oxides, and the results indicated that the adsorption characteristics of roxarsone and arsanilic acid were similar, but the adsorption efficiency on the surface site basis was about three times lower for Al₂O₃ than for FeOOH. Liu et al [44] studied on the treatment of the waste containing arsenic generated in the production process of arsenical pesticide intermediates, the organic arsine waste was oxidized from arsenite to arsenate, and the mass ratio of organic arsine waste and oxidizer was 1:0.2, solid residue and waste water were separated. The waste water was treated before it was discharged to municipal waste water plant, the residue was disposed at landfill site, and the process achieved the purpose of harmless handling. Flavomycin and bacitracin zinc are two relative safe feed additives which are no drug resistance, no residue and no pollution, and can reduce the consumption of roxarsone, can maintain or increase their growth promoting effect, and reduce the pollution to environment because of adding roxarsone. The arsenic is discharged in the form of As^{5+} by environmental regulation, this will be conducive to the removal of organic arsine of livestock and poultry manures by anaerobic digestion, and then reduce or eliminate environmental arsenic pollution caused by organic arsine additives. Though the arsenic in environment can not be biodegraded as organic contaminants, its bioavailability can be affected by microorganisms via the processes oxidation and reduction, absorption and desorption, methylation and demethylation, and precipitation and dissolution and so on, and thereby, its toxicity could be reduced, and the arsenic-contaminated environment could be remediated [45].

In recent years, many countries have restricted the application of organic arsine preparations. The European Union and Japan announced that the arsenic feed additives were forbidden. Food and Drug Administration (FDA) had cancelled the use of three kinds of arsenic (roxarsone, arsanilic acid and carbarsone) in veterinary medicine license in 2013, but nitrosone is still permitted to use in the United States. In 2004, Tyson food companies, America's largest producer of poultry, made a statement stop using arsenic feed additives. MacDonald, the world consumption giant of poultry, also required their suppliers must not use arsenic feed additive to feed poultry. The world's largest pharmaceutical company, Pfizer Company, had suspended sales roxarsone in the United States. In addition, the complete health quality inspection system and the waste disposal ordinance have been established in the United States for the management of meat and egg products fed arsenic preparation. However, the policy and system in the aspects are relative absent in China. As a result, the various departments in China should seriously implement the feed and feed additives regulations, and earnestly perform the feed management and supervision responsibilities, strictly control the production and application of organic arsine preparations in feed so as to ensure the quality and safety of feed products. The food and feed hygiene standards in China made strict rules: the allowable residue limit of arsenic in animal products like meats, eggs, liver and kidney was 0.5 mg/kg and 2 mg/kg respectively. The world health organization (WTO) stipulated that the content of arsenic in the food should be less than 0.1 mg/kg, and the maximum residue limit of inorganic arsenic in the powdered milk was 0.25 mg/kg. In view of Chinese 1.3 billion populations' the huge demand for pork and the stage of development of animal husbandry, it is very difficult to forbid completely the arsenic preparations at short notice, and therefore we should follow the principle of gradual improvement and gradually ban the use of organic arsine preparations as additives.

CONCLUTION

Organic arsine preparations such as arsanilic acid and roxarsone as the growth promoting agent of livestock and poultry have gained some economic benefits in the short time. But their toxicities and side effects and the pollution to environment are more than their beneficial functions. The rise of arsenic content in the ecosystem not only endangers livestock and poultry but also directly relates to the safety of human beings. No matter from the perspective of toxicity or from the perspective of ecological system, the organic arsine preparations have brought huge and lasting harm. The pollution problem of arsenic has become a social focus. With the improvement of living standard, people pay more and more attention to the life safety and food safety, and the consciousness of environmental protection has been strengthened. Green and safe food will become the mainstream of people consumption, the prohibition of use of the organic arsine preparations will become the inevitable choice of the feed industry.

The number of breeding of livestock and poultry in China is very large, if the organic arsine preparations are applied year after year, the large amounts of feces containing arsenic will be discharged into the environment, so it is urgent to ban the use of the arsenic preparations as the growth promoting agent of livestock and poultry. Weigh the advantages and disadvantages, in order to strengthen the competition ability of our country animal products entered the international market and protect our living environment, we should ban the use of the arsenic preparations as feed additives as early as possible. If the use of arsenic preparations is unlimited, it is bound to affect the sustainable development of animal husbandry in our country, and cause irreparable damage to the ecological environment. We call on forbidding the production and application of the organic arsine preparations in feed industry as soon as possible.

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