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The Effect of Ingestion of Diatomaceous Earth in White Rats: A Subacute Toxicity Test[1]

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Diatomaceous earth, the siliceous remains of unicellular and colonial algae, has been studied quite extensively in relation to occupational disease in the diatomite mining and processing industry. Among the first to recognize the problem were Legge and Rosencrantz (1932), who studied pneumoconiosis in a California plant that processed diatomaceous earth. Smart and Anderson (1952) observed that inhalation of diatomaceous earth may produce a distinct lesion characterized by linear fibrosis; furthermore they concluded that diatomaceous earth from freshwater origin appeared to be less toxic than that originating from the marine diatom. Luton *et al.* (1956) observed that amorphous silica was only moderately, if at all, harmful when inhaled. When this amorphous form was heated to approximately 1250°C. cristobalite, a crystalline form, was produced which was as dangerous as quartz. Vigliani and Pecchiani (1949) reported nodular reactions in the peritoneal cavities of guinea pigs injected with calcined diatomite, whereas the peritoneal cavities of guinea pigs injected with crude diatomite showed little change.

Diatomaceous earth is currently being used in insect control, particularly in granaries. The grain so treated will have a residue, which according to the Food and Drug Administration is subject to the Pesticide Amendment to the Federal Food, Drug, and Cosmetic Act. However, little is known as to the tissue damage caused by the mechanical and chemical activity of the diatom particle over a period of time.

The purpose of the toxicity test was to determine the possible damage and to ascertain the maximum concentration that would not produce tissue damage. It was suggested that 5, 3, and 1% diatomaceous earth be used in the diet of test animals and that the test be carried out over a 90-day period. Furthermore, if no damage occurred at the 5% level, only spot checks would be done with animals fed the lower concentration.

The following is a summary of the report submitted to the Division on Pharmacology, Toxicity Branch of the Federal Food and Drug Administration.

MATERIAL AND METHOD

Weanling white rats of the Wistar strain, average weight 50 g, were used. These animals were distributed into four groups, each containing 15 males and 15 females. Group I was designated as the group that would receive 5% diatomaceous earth added to the diet; group II would receive 3%; group III, 1%; and group IV the controls

[1] This research was performed for the Phoenix Gem Co., Inc. through Arizonia State University Foundation.

received the diet without the added test material. The rats were allowed free access to the food throughout the experiment.

The diatomaceous earth[2] was of freshwater origin. Four 50-pound bags were selected at random from the stockpile, and samples were taken from each bag in order to determine the particle size distribution and chemical composition.[3]

To ensure constant concentration, the diatomaceous earth was mixed with water to obtain a thin paste and added, a small quantity at a time, to preweighed feed in a tumbling apparatus. It was possible to obtain uniform distribution of the test substances on the pellets. These pellets were dried prior to use.

Weekly weight changes were recorded for each rat. At the end of the 90 days, the test was terminated and the animals were killed. Autopsies were performed and histologic sections were made from the following organs: stomach, small and large intestines, liver, kidneys, spleen, lung, urinary bladder, adrenal glands, mesenteric lymph nodes, and testes or ovaries. Organ weights were recorded for the livers, kidneys, and spleens; in addition, organ weight:body weight ratios were calculated for each animal. Parts of these same organs were analyzed for residual silica.

For histologic examinations, the tissues were fixed in 10% formalin and stained with Weigert's hematoxylin and eosin stains.

RESULTS

Analysis of Particle Size Distribution

The largest-sized particles in the test material was 0.64 mm. Ninety per cent were smaller than 0.1 mm; of these 55% were smaller than 0.012 mm. The smallest size of the test material was 0.00046 mm.

Chemical Analysis

Spectrographic analysis of the test material is tabulated in Table 1. The major components were in the form of oxides.

Analysis for Residual Silica

Organ analysis for silica in the liver, kidney, and spleen of the 5% level animals and controls are summarized in Table 2. These results are the averages for fifteen animals per group. No analysis were made for the lower two concentrations.

Physical Analysis

Summary of organ weights of livers, kidneys, and spleens are tabulated in Table 3. The average weights for fifteen animals were used in each average grouping. Table 4 reflects the average organ weight to body weight ratio of the 5% level test group and controls. No averages were made on either the 3 or 1% levels.

Weight Changes

The average weekly weight change of animals fed 5% diatomaceous earth over the 90-day period is summarized in Fig. 1. The graph reflects the average weight gain,

[2] Perma Guard, trademark of Phoenix Gems Inc., Phoenix, Arizona. From San Manuel lacustrine deposits.

[3] All chemical analyses were performed by the Arizona Testing Laboratories, Phoenix, Arizona.

TABLE 1

Spectrographic Analysis of Diatomaceous Earth Used in Toxicity Test

Components	Percentage
Major elements (oxides)	
Silicon	85.164
Aluminum	3.9
Sodium	5.0
Iron	2.0
Magnesium	1.0
Minor or trace elements	
Boron	0.06
Manganese	0.002
Gallium	0.002
Calcium	0.84
Vanadium	0.002
Copper	0.01
Titanium	2.0
Zirconium	0.01
Strontium	0.01
	100.000

TABLE 2

The Analysis of Residual Silica from Test Animals As Compared to Controls

Animal	Per cent silica
Liver	
Control males	0.002
5% males	0.002
Control females	0.002
5% females	0.002
Kidney	
Control males	0.002
5% males	0.002
Control females	0.002
5% females	0.001
Spleen	
Control males	0.002
5% males	0.003
Control females	0.003
5% females	0.003

TABLE 3

Comparison of Average Organ Weights in Grams of Control Animals to the 5% Test Group (15 Animals per Group)

	Control animals		5% test animals	
Organ	Male	Female	Male	Female
Liver	15.46	9.68	14.60	11.05
Kidney	1.58	1.13	1.43	1.20
Spleen	1.46	1.10	1.42	1.01

TABLE 4
RATIO OF ORGAN WEIGHT TO BODY WEIGHT OF THE 5% TEST ANIMALS AS COMPARED TO THE CONTROL ANIMALS

Animal group	Organ weight : body weight
Control males	
Liver	0.0480
Kidney	0.00544
Spleen	0.00520
5% males	
Liver	0.0481
Kidney	0.00532
Spleen	0.00520
Control females	
Liver	0.0521
Kidney	0.00531
Spleen	0.00542
5% females	
Liver	0.0520
Kidney	0.00538
Spleen	0.00420

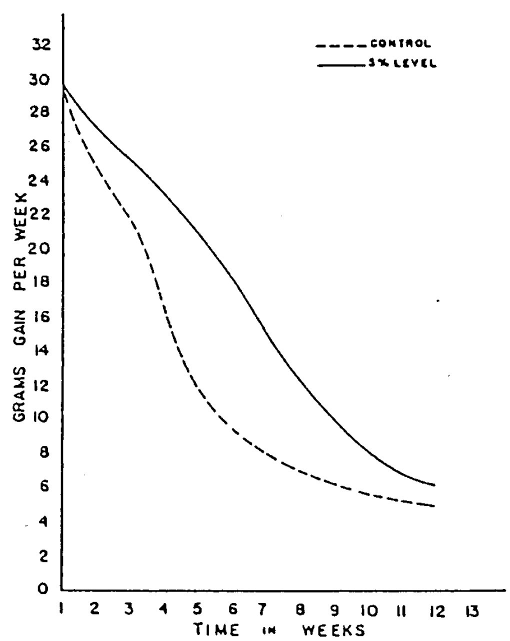

FIG. 1. Average weight gain in grams per week of test animals as compared to controls (30 animals were used in each group).

(combined values for males and females) in grams per week. It was noted that after the first week, the animals fed 5% diatomaceous earth had a higher weight gain than the control animals. The maximum weight gain differential was reached during the sixth week. The following weeks, although the test animals continued to gain more rapidly than the controls, the weight gain differential became progressively smaller.

At the end of the test, the test animals were still gaining more than the controls. Projection beyond the 90-day period indicates that the weight gains would possibly be equal sometime between the fourteenth and fifteenth week. No accurate averages were made on the lower two levels: however it suffices to estimate that those animals on the 3% diet had a weight gain similar to that of animals at the higher level, while those animals on the 1% level had weight gains similar to those of controls.

Histologic Analysis

Complete histologic studies were made on the 5% group; in addition, six animals from both the 3% and the 1% groups were examined for comparative purposes.

No differences were observed in sections from the various organs of the 5% group and comparable sections of animals fed no diatomaceous earth (Figs. 2 and 3c). In both the 5% and control groups there were observed an equal number of animals which had a form of lobar pneumonia. In addition, inner zonal fatty degeneration was noted in the livers of five control animals and six animals on the 5% diet.

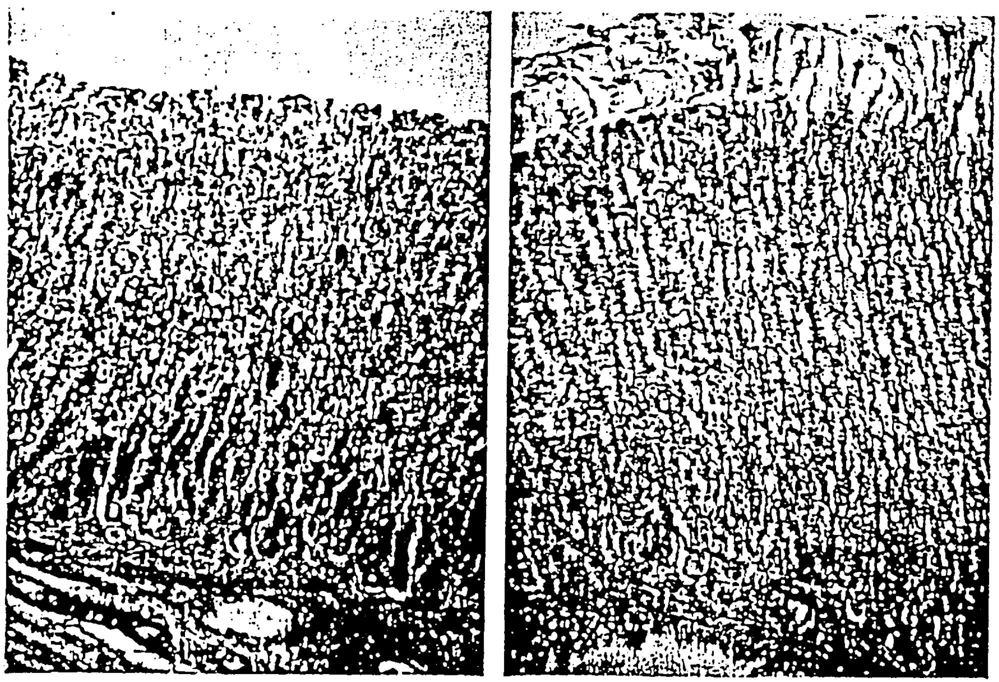

FIG. 2. Fundic region of the stomach of a test animal fed 5% diatomaceous earth (left) compared with comparable area from a control (right). No mechanical damage is evident that is due to the action of the diatomite particles. Hematoxylin and eosin stains. Magnification: × 125.

Of animals fed the lower two concentrations, the sections of the various organs showed no histologic changes when compared with similar areas from the controls.

DISCUSSION

In spite of the hardness of diatomaceous earth, no mechanical damage was observed from histologic sections taken from animals fed 5% of this material in their diet. This is significant in view of the fact that those animals were receiving diato-

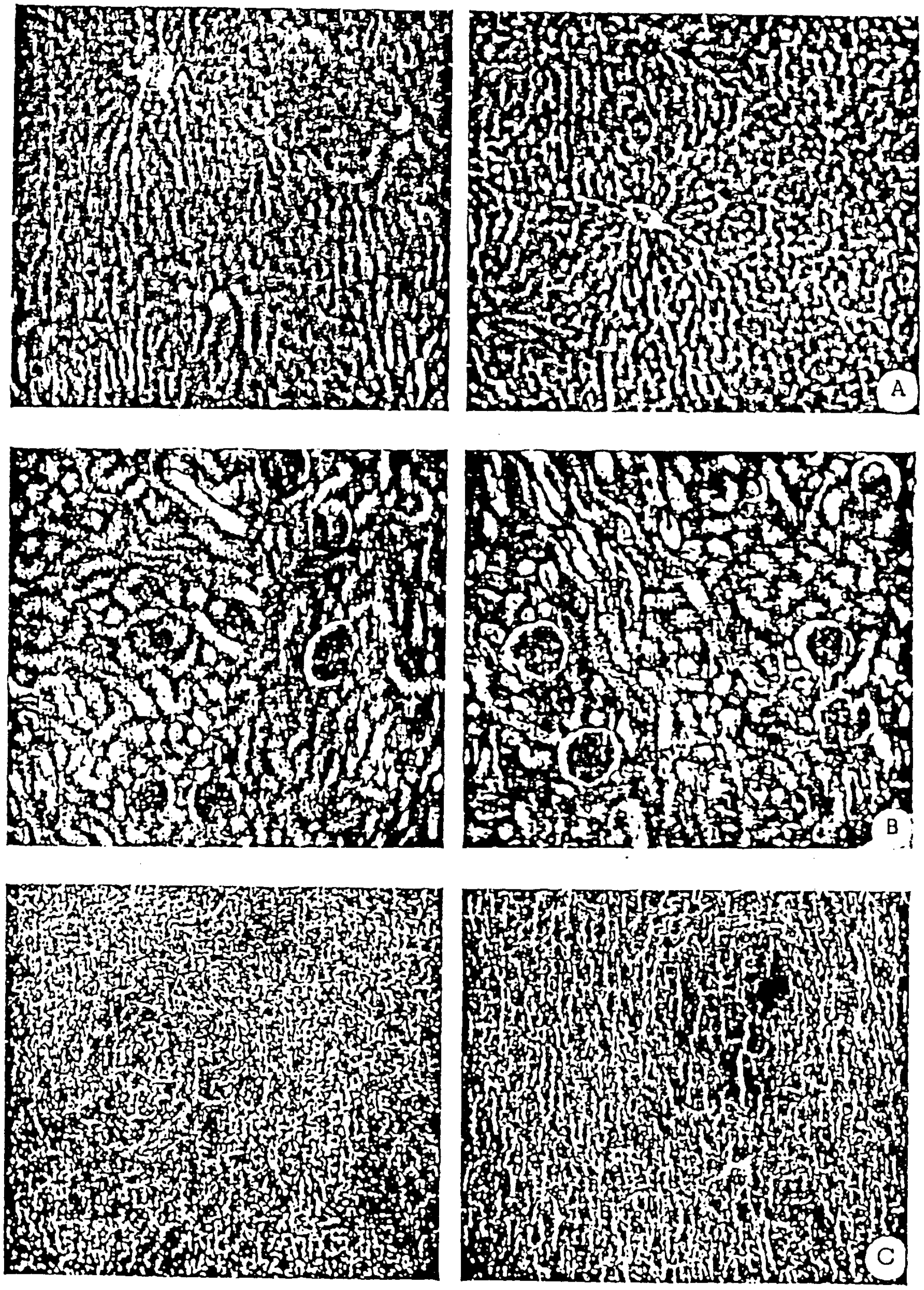

FIG. 3. Comparison of sections of (A) liver, (B) kidneys, and (C) spleen of the 5% level test animals (right) as compared to animals kept on a control diet (left). No apparent damage resulted from the indirect action of diatomaceous earth on these organs. Parts of the same organs were analyzed for residual silicon. Hematoxylin and eosin stains. Magnification: × 125.

maceous earth continually over a 90-day period. The lack of observable lesions (Fig. 2) is believed to be due to several factors. Examination of particle size distribution shows lack of coarse particles that would cause abrasive action. The largest particle size was approximately 0.64 mm; 90% of the remaining distribution was less than 0.12 mm. The second factor was the mode of administration. The diatomite particles were contained on the pellets and the white rats were allowed to feed ad libitum; consequently only a small amount of silica was taken in at any time with the food. Examination of the stomach content showed complete mixing of the test material with the food.

The lack of histologic changes other than at the primary site of action indicated that no toxic chemical absorption had occurred (Fig. 3). It was possible for the silica component, in an alkaline medium such as was present in the small intestines, to be converted into a relatively soluble silicic acid. Even if the amount per day was extremely small, the combined action over a 90-day period could be significant. Once in the circulatory system a drop in pH to 7.4 would precipitate the silica. The precipitated form could be in the crystalline state. If this did occur, necrotizing lesions would be seen because the crystalline form would be as dangerous as quartz. If the precipitate were in the amorphous state no reaction was to be anticipated since no changes occurred in the intestinal tract. To check further on the change in silica count, chemical analyses for residual silica were made from livers, kidneys, and spleen (Table 2). The results indicated no increase in the silica content of these animals. No analyses were made of these organs in the lower two levels.

Analysis of the organ weights and organ weight : body ratios further substantiate the nontoxicity of the diatomite particles (Tables 3 and 4). These animals were in an active growth stage as weanlings. Any toxic material would possibly affect the organ growth of the liver, kidney, and spleen. There was no evidence of atrophy or hypertrophy of these organs.

Aluminum, the next important compound, is apparently nontoxic according to the results of a study made by Campbell *et al.* (1956). The other compounds present were either in too small a concentration or could be utilized by the animal.

It was interesting to note the comparative weight changes. Not only did the test animals gain weight, but from the onset of the test the rate of growth exceeded that for the controls. The maximum rate change occurred during the sixth week of the test. At that time, the average weight change of the test animals over the controls were 45 g (Fig. 1). The weight differential after the sixth week continued, but on a decreasing weekly increment. Had the test been continued beyond 90-days, the weight changes should have been approximately the same for both groups during the fourteenth week.

One can only speculate as to the cause of the increase of weight of the test groups over the controls. One possibility relates to particle size. As particle size decreases, relative surface area increases, resulting in a high surface action. Owing to the adsorbability of diatom particles and the size distribution in this test, the highly active surface could adsorb food, creating not only a further increase in surface area for enzyme reaction, but allowing longer time of action for more complete digestion. Hence, there would be more enzyme product for the animal to utilize. A second possibility is the presence of minor or trace compounds (Table 1). Elements such as sodium, iron, magnesium, manganese, calcium, and copper are necessary for either general metabolism or special cellular metabolism. The supplement, in conjunction with the high surface area and adsorptive property of diatomaceous earth, may account for this weight change. The graph (Fig. 1) also indicates that the abnormal weight changes are important in the fairly young stage of development. The weight differences were not reflected, however, in an increase of fat deposits.

From the results of this test, there is no evidence of toxic changes due to the ingestion of diatomaceous earth.

Summary

The effects of 5% diatomaceous earth in the diet fed to white rats over a 90-day period indicated no mechanical or chemical damage to the tissues.

There was no significant increase in the percentage of residual silica in liver, kidneys, and spleens of the test animals.

Comparison of weight changes in the 5% group with the control showed a greater weight gain in the 5% test group.

Histologic sections of animals fed 3% and 1% diatomaceous earth also showed no damage.

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ケイソウ土の毒性試験の概要

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薬剤の概要

ケイソウ土(Diatomaceous Earth、商品名：コクゾール®)は、日本では三共㈱が1987年から玄米における病害虫防除効果試験を実施し、1988年に90%粉剤が農薬登録され、現在に至っている。

本剤は食品添加物である天然のケイソウ土を有効成分としており、その防除作用は機械的致死作用である。

本剤（二酸化ケイ素として）の化学構造及び物理的化学的性状等は次に示すとおりである。

化学式：Silicon Dioxide

構造式：O=Si=O

分子式：SiO_2

分子量：60.08

外　観：白色

比　重：2.1

以下、本剤の登録取得等に際して実施した安全性評価のための各種毒性試験を取りまとめて報告する。

急性毒性試験

ラット及びウサギに対する種々の投与経路における急性毒性試験の結果は表1に示すとおりである。

表1　急性毒性試験結果

検　体	動物種	投与経路	LD50(mg/kg)	実施機関(年度)
90%粉剤	ラット	経　口	♂♀共に >5000	Applied Biological Sciences Laboratory (1984年)
	ウサギ	経　皮	♂♀共に >2000	
	ラット	吸　入	♂♀共に >4.8(mg/l)	

刺激性試験

1．眼一次刺激性試験

90%粉剤の眼に対する刺激性をウサギを用いて検討した。

片眼を処理眼、他眼を無処理対照として、検体100mgを投与し、刺激性の評価を角膜、虹彩及び結膜について行なった。また、洗眼群も設定した。

その結果、非洗眼群では眼に対し非常に軽度な刺激性が認められたが、投与2日目までには消失した。また、洗眼群では刺激性変化は認められなかった。

(Applied Biological Sciences Laboratory、1984年)

2．皮膚一次刺激性試験

90%粉剤の皮膚に対する刺激性をウサギを用いて検討した。

検体500mgを蒸留水で湿らせ、刈毛した動物の背部皮膚4ケ所(2ケ所；非擦過、2ケ所；擦過、各2.5cm四方)にそれぞれ24時間塗布し、塗布部分における紅斑、痂皮及び浮腫等の刺激性変化の有無を観察した。

その結果、皮膚に対し刺激性は認められなかった。

(Applied Biological Sciences Laboratory、1984年)

要　約

ケイソウ土の安全性評価のため、各種毒性試験を実施した。

ケイソウ土の急性毒性はいずれの投与経路においても低毒性であった。刺激性試験では、眼に対して非常に軽度な刺激性が認められたが、洗眼群では刺激性は認められなかった。また、皮膚に対しては刺激性が認められなかった。

以上の結果から、本剤は定められた使用規準を遵守することにより十分に安全性を確保できる有用な農薬の1つであると考えられる。

問合せ

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