EVALUATION OF THE HEALTH ASPECTS OF CARBON DIOXIDE
AS A FOOD INGREDIENT

1979

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office 
Federation of American Societies 
for Experimental Biology 
9650 Rockville Pike 
Bethesda, Maryland 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administra-
tion (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assess-
ments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administra-
tion, after announcement in the Federal Register, and opportu-
nity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final con-
clusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Soci-
eties for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

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I. INTRODUCTION

This report concerns the health aspects of using carbon dioxide as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1974.* To assure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of April 6, 1979 (44 FR 20797-20800) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using carbon dioxide as a food ingredient. The Select Committee received no requests for such a hearing on carbon dioxide.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-241 959/6) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluation of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on carbon dioxide and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

Carbon dioxide (CO₂) occurs widely in nature as a colorless, odorless gas, which dissolves in water in about a 1:1 ratio by volume. It has a molecular weight of 44.01, and 1 liter at standard temperature and pressure weighs 1.977 g (3,4). Carbon dioxide comprises about 0.03 percent of the normal ambient air on earth; one liter of air contains about 0.6 mg carbon dioxide. When compressed and allowed to expand rapidly, the solid form (dry ice) is readily produced, having a temperature of -78°C. When added to water under pressure, "soda water" is produced which effervesces as the pressure is released. The pleasant and reputed medicinal properties of effervescent waters date back to Greek and Roman days, but the carbonated beverage industry began only in the nineteenth century (5,6).

Carbon dioxide is obtained industrially as a byproduct in the manufacture of lime during the burning of limestone, or from the combustion of coke or other carbonaceous materials. In the United States, large amounts are derived as a byproduct of the yeast fermentation of glucose in the production of ethyl alcohol (7). The Encyclopedia of Food Technology (4) states that the greatest use of gaseous carbon dioxide is for the production of carbonated beverages. The carbonation of beverages has increased because of the pungent taste it imparts and the bubbling effect as the fluid is poured from the container.

Specifications for food grade carbon dioxide do not appear in the Food Chemicals Codex (7a) and the United States Pharmacopeia (8) merely states that not less than 99.0 percent of CO₂ must be present. Carbon dioxide is listed in the Code of Federal Regulations (2) as a multiple purpose GRAS additive for foods [21 CFR 182.1240] and for animal feeds [9 CFR 582.1240]. It is also approved as a cooling agent in the preparation and packaging of meat products [9 CFR 318.7] (9). As an ingredient of soda water, carbon dioxide must be added to potable water in an amount "not less than that which will be absorbed by the beverage at a pressure of one atmosphere and at a temperature of 60°F" [21 CFR 165.175]. It is authorized as a propellant and aerating agent for foamed or sprayed food products [21 CFR 173.345; 173.360] and is used for this purpose in certain dairy product analogs, such as whipped cream (10).

Carbon dioxide has certain advantages over nitrogen- or vacuum-packaging of coffee, bacon, and cheese (11). In addition to excluding oxygen, carbon dioxide also inhibits the growth of many aerobic and some anaerobic microorganisms. Upon solution, it produces a weak acid (carbonic acid). When used over food items wrapped in plastic film, the carbon dioxide is readily absorbed to produce a partial vacuum, which molds the transparent film to the product (12).
Carbon dioxide added directly or through fermentation produces effervescence in alcoholic and nonalcoholic beverages and also serves as an acidifying agent (4). It is used in Denmark as a preservative without restriction in apple juice and berry mist; and in France, in fruit and vegetable juices, lemonade, sparkling wine, aerated waters, and sodas (13).

In its solid form, carbon dioxide has long been used in the storage and transport of frozen foods. It may be mixed with powdered foods to attain rapid freezing (14). For many foods, however, it is an ineffective freezing agent because of difficulties in establishing adequate contact between solids.
A National Research Council (NRC) subcommittee (15) surveyed manufacturers in 1970 concerning the level of addition of the GRAS substances to foods and estimated their possible average daily intake. Based on information supplied by those manufacturers who reported adding carbon dioxide to at least one food in a category, weighted means were calculated for its usual and maximal addition to foods in the category. Weighted means of the usual level of addition of carbon dioxide are given in Table I. It should be noted that these weighted means do not express the highest percentage of this substance added by any manufacturer; they do not indicate that all foods in a category contain added carbon dioxide; and they do not necessarily coincide with the levels added by any one manufacturer. Also listed in Table I are the categories of foods to which carbon dioxide is added and the purpose of these additions.

The NRC subcommittee has estimated possible average daily intakes of carbon dioxide for various age groups from data collected by the Market Research Corporation of America (Chicago, Illinois) on the mean frequency of eating foods by food category, data on mean portion size of foods in those categories from the U.S. Department of Agriculture, and the assumption that all food products within a category contain carbon dioxide at the levels shown in Table I (15). These calculations indicate a possible average intake of persons over 2 years of age to be about 1.7 g daily. However, because of various factors discussed in Section XI of its report, the NRC subcommittee has recognized that in most cases, this approach is likely to overestimate the actual intakes, often by considerable margins.

An alternative estimate of the intake of carbon dioxide may be made from the consumption data presented in Table II (15). It is apparent that the intake estimated in this manner, 270 mg daily, is substantially less than that obtained by the former calculation (1.7 g). The Select Committee believes that even this lower value exaggerates the average amount of carbon dioxide actually ingested. Much would escape from the food, especially from carbonated beverages, before consumption. Carbon dioxide is generally added to beverages under 2 to 5 atmospheres pressure with a corresponding increase in solubility (16). Upon opening the container, the beverage is exposed to normal atmospheric pressure and most of the dissolved carbon dioxide escapes. Individuals drinking large quantities of carbonated beverages may ingest several grams of carbon dioxide daily. How much of this would be expelled subsequently by eructation is not known.
<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean percent</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>&lt;0.01</td>
<td>Processing aid, propellant, aerating agent</td>
</tr>
<tr>
<td>Processed fruits, juices, &lt;0.01</td>
<td></td>
<td>Antioxidant</td>
</tr>
<tr>
<td>and drinks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td>0.20</td>
<td>Processing aid</td>
</tr>
<tr>
<td>Beverages, nonalcoholic*</td>
<td>0.70</td>
<td>Flavor enhancer, flavoring agent, propellant, aerating agent</td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td>1.44</td>
<td>Flavor enhancer, propellant, aerating agent, surfactant</td>
</tr>
<tr>
<td>Dairy product analogs</td>
<td>0.12</td>
<td>Propellant, aerating agent</td>
</tr>
</tbody>
</table>

*Seven manufacturers reported. For all other categories, three or fewer reported.
### TABLE II

Carbon Dioxide Used by the Food Processing Industry and Calculated Per Capita Daily "Intake" (15)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative amounts used 1970/1960</th>
<th>Total used (1970) kg</th>
<th>Per capita daily intake mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>1.85</td>
<td>20,200,000</td>
<td>270</td>
</tr>
</tbody>
</table>

1. Based only on reports from those respondents to the National Research Council survey who submitted information for both 1960 and 1970 (15).

2. Total usage is based on amounts added to foods as reported to NRC and Flavor and Extract Manufacturers' Association, which was estimated to represent about 60 percent of the actual usage, recalculated to 100 percent.


4. The poundage reported in a resurvey made in 1975 (63) was about the same as that reported in 1970.
IV. BIOLOGICAL STUDIES

Carbon dioxide performs many vital functions in the body. Among other actions, it is a principal regulator of respiration; it assists in the liberation of oxygen to the tissues; it is essential for the maintenance of the acid-base balance; it exerts profound effects on the central nervous and cardiovascular systems; it is a powerful cerebral vasodilator; it influences the electrolyte balance in the tissues; it is a key component of numerous metabolic processes, both anabolic and catabolic; together with water, it is the end product of carbohydrate and fat combustion; and it combines with ammonia and adenosine triphosphate to produce carbamoyl phosphate, an important intermediate in the synthesis of urea and pyrimidines (17-19).

Absorption and distribution

Under normal circumstances, carbon dioxide is a gas and enters the body through the lungs. For this reason, little attention has been paid to its absorption from the gastrointestinal tract. The Select Committee was unable to locate any report of the absorption of ingested carbon dioxide. In neutral solutions, at standard temperatures and pressures, about 1 ml (2 mg) of the gas will dissolve in each ml of solution. In alkaline solutions, the carbon dioxide is converted to carbonates and bicarbonates and it is in this form that any ingested carbon dioxide would likely be present in the intestinal tract. The daily average intake of carbon dioxide in foods would be equivalent to approximately 1 g daily of sodium bicarbonate or carbonate and would represent only a very small fraction of the amounts of these compounds in normal diets (20,21).

In addition to the carbonates and bicarbonates normally ingested in foods, much larger quantities are often taken as antacids. The U.S. Dispensatory (3) indicates the usual dosage range of sodium bicarbonate to be from 300 mg to 16 g daily. Kirchner and Palmer (22) reported a young patient who had received 32.0 kg sodium bicarbonate over a period of 20 months (about 50 g daily) for treatment of duodenal ulcer, without significant effect on the acid-base balance, urea clearance, hemoglobin, or red- and white-blood-cell counts.

Carbon dioxide is constantly produced in the body as a result of the numerous metabolic reactions involving carbon-containing compounds. The amount produced depends primarily upon the activity of the individual. Under resting conditions, a 70 kg man will utilize about 245 ml of oxygen per minute and exhale about 215 ml carbon dioxide (respiratory quotient 0.88) (23). This represents the production and excretion of over 500 g of carbon dioxide daily. Under conditions of moderate or heavy activity, this amount may increase severalfold.
The carbon dioxide diffuses from the tissues into the surrounding capillaries and is carried by the blood to the lungs (17). The total carbon dioxide content of the venous blood is usually 55 to 60 ml per 100 ml blood, only a small part of which is in solution. The dissolved carbon dioxide is slowly hydrated to form carbonic acid, a process catalyzed in the erythrocyte by carbonic anhydrase. At the alkaline pH of the blood, the carbonic acid is rapidly converted to bicarbonate and it is in this form that most of the carbon dioxide is transported in the blood. Some of the carbon dioxide also reacts with aliphatic amino groups to form carbamino-compounds. Only small amounts of carbon dioxide are bound in this manner to plasma proteins, but significant quantities of carbamino-compounds are bound to hemoglobin and especially to the reduced hemoglobin in venous blood. Each liter of venous blood transports about 1 g of carbon dioxide in the various forms and discharges approximately 75 mg during each passage through the lungs. The distribution of carbon dioxide in blood is illustrated in Table III.

The partial pressure of carbon dioxide in the pulmonary capillary blood (46 mm Hg) is greater than that in the alveolar air (40 mm Hg). This concentration gradient allows the gas to diffuse across the alveolar membrane into the alveolar air and to be exhaled (17).

Physiological actions

Respiratory effects. Because of its solubility in tissue fluids, carbon dioxide readily diffuses through tissue membranes and acts upon the various chemoreceptors in the body (24). These include receptors in the aortic arch, the carotid body, and the medulla. The central chemoreceptors in the medullary respiratory center are especially important in respiratory control. They are extremely sensitive to minute changes of hydrogen ion and carbon dioxide concentrations. A delicate feedback mechanism maintains the concentrations within very narrow limits. When the carbon dioxide tension in arterial blood increases, the respiratory ventilation increases, and when the tension falls below normal levels, respiration decreases. A rise of only 2 to 3 mm Hg in the arterial carbon dioxide tension may cause a doubling of the ventilation rate. Dripps and Comroe (25) reported more than a tenfold increase in respiratory minute volume of normal male subjects inhaling 10.4 percent carbon dioxide: from 7 liters per minute with ambient air to 76.3 liters per minute with the carbon dioxide mixture. Within 3 minutes after the subjects had returned to ambient air, their respiratory rates returned to normal. The carotid chemoreceptors also respond to increased carbon dioxide tension of the blood, but they are distinctly less sensitive than the cells of the medullary center (26).

Cardiovascular effects. Inhalation of carbon dioxide increases cardiac output, heart rate, and blood pressure (18). Healthy men breathed 7.6 and 10.4 percent carbon dioxide for 7.4
<table>
<thead>
<tr>
<th>CO₂ distribution</th>
<th>Arterial mmoles</th>
<th>Venous mmoles</th>
<th>Difference mmoles</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CO₂ per 1 whole blood</td>
<td>21.53</td>
<td>23.21</td>
<td>1.68</td>
<td>73.9</td>
</tr>
<tr>
<td>Total CO₂ in plasma (600 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as dissolved CO₂</td>
<td>15.94</td>
<td>16.99</td>
<td>1.05</td>
<td>46.2</td>
</tr>
<tr>
<td>as bicarbonate</td>
<td>0.71</td>
<td>0.80</td>
<td>0.09</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>15.23</td>
<td>16.19</td>
<td>0.96</td>
<td>42.2</td>
</tr>
<tr>
<td>Total CO₂ in erythrocytes (400 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as dissolved CO₂</td>
<td>5.59</td>
<td>6.22</td>
<td>0.63</td>
<td>27.7</td>
</tr>
<tr>
<td>as bicarbonate</td>
<td>0.34</td>
<td>0.39</td>
<td>0.05</td>
<td>2.2</td>
</tr>
<tr>
<td>as carbamino-CO₂</td>
<td>4.28</td>
<td>4.41</td>
<td>0.13</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>1.42</td>
<td>0.45</td>
<td>19.8</td>
</tr>
</tbody>
</table>

*Adapted from reference 17.
and 3.8 minutes, respectively. The respective increases were 30.8 and 33.4 mm Hg for systolic pressures; 22.2 and 25.0 mm Hg for diastolic pressures; and 17 and 16 beats per minute for heart rates (25). Kety and Schmidt (27) also reported an increase in blood pressure in subjects exposed to 5 or 7 percent carbon dioxide for 15 to 30 minutes. The most striking change was an average increase of 75 percent in the cerebral blood flow.

Electrocardiographic changes were noted among healthy men receiving 6 percent carbon dioxide for 6 to 8 minutes. A significant decrease (19.1 percent) was noted in the amplitude of the QRS complex among the older subjects (mean age 60.9 years) and a decrease of 11.6 percent among the younger men (mean age 23.3 years) (28). Frequent and marked changes were noted when 30 percent carbon dioxide and 70 percent oxygen were given to male subjects (25 to 48 years of age) for an average of 38 seconds. In 12 of the 17 subjects, some abnormality or nodal activity resulted, including extra systoles, premature auricular and nodal beats, and auricular and supraventricular tachycardia (29). Friedlander and Hill (30) also reported extra systoles in 5 of 37 patients exposed to 30 percent carbon dioxide and 70 percent oxygen for 50 to 52 seconds.

Neurological effects. The inhalation of low concentrations (5 to 10 percent) of carbon dioxide depresses the excitability of the cerebral cortex whereas high concentrations may reverse this effect and so increase cortical excitability that convulsions may occur (18). Young psychiatric patients breathing 30 percent carbon dioxide in oxygen lost consciousness in 24 to 28 seconds (14 to 16 breaths) (30). At concentrations of 10 to 15 percent of the gas in oxygen, neurologic signs (including psychomotor excitation, myoclonic twitches, and eye flickering) may occur within 2 minutes (31). Within 4 minutes of exposure to 10.4 percent carbon dioxide, most of 31 young subjects reported one or more symptoms of headache, sweating, dizziness, mental clouding, faintness, and restlessness (25).

Schaefer (32) found slightly decreased psychomotor performance in subjects after 15 minute exposure to 7.5 percent carbon dioxide. Weitzman et al. (33) performed a variety of visual function tests on a healthy young man exposed to gradually increasing carbon dioxide concentrations from 0.03 to 3.0 percent for 15 hours daily for 6 days. No decrement in function could be detected except for slight decreases in night vision sensitivity and color sensitivity for green. Schäfer (34) exposed five volunteers to 3 percent carbon dioxide for 8 days in an environmental chamber. Excitatory effects and some euphoria were noted during the first day, followed by significant performance decrements for the rest of the test period. However, Glatte et al. (35) subjected seven young male volunteers to the same concentration of carbon dioxide (3 percent) for 5 days and could detect no decrement in any of the psychological and psychomotor tests employed.
Acid-base balance

Several investigators have studied the effects of exposure to moderate levels of carbon dioxide, as might occur during submarine duty. Naval investigators (36-38) studied 23 men confined in a submarine and exposed to 1.5 percent carbon dioxide for 42 days. An uncompensated respiratory acidosis was noted during the first 23 days followed by a compensatory phase during the remaining exposure period. The period of uncompensated acidosis was characterized by decreases in blood and urinary pH, in urinary bicarbonate and pulmonary carbon dioxide excretion, and in plasma calcium and inorganic phosphate.

During the compensatory phase (days 24 to 42) all these indices were reversed. The blood pH returned to normal; the urinary pH rose; the excretion of pulmonary carbon dioxide and of urinary bicarbonate increased, as did the plasma levels of calcium and inorganic phosphates. At a higher carbon dioxide concentration (3 percent) similar acidic changes were noted, but compensatory responses to the acidosis occurred much earlier (within 3 days) (39).

Acute toxicity

All available toxicity studies refer to inhalation effects. The United States Occupational Standard for carbon dioxide allows a concentration of 5000 ppm (9000 mg per m³) in workroom atmospheres (40). Anesthetized dogs were exposed to a mixture of 30 percent carbon dioxide and 70 percent oxygen for 2 hours, followed by a 2-hour exposure to 40 percent carbon dioxide (41). When they were abruptly returned to air, 11 of 15 developed ventricular fibrillation and died within 2.5 to 10 minutes. Cardiac arrhythmias appeared in the four surviving dogs. However, two dogs similarly exposed but gradually returned to air breathing survived with no signs of cardiac dysfunction.

Rats were exposed to gas mixtures, each containing 21 percent oxygen, but with carbon dioxide concentrations varying from 10 to 50 percent (42). All rats exposed to 50 percent carbon dioxide died within 6 hours and all those receiving 25 percent within 36 hours. Cerebral depression was produced at concentrations as low as 20 percent. Rats dying from carbon dioxide exposure always showed pulmonary injury. The lungs were edematous and filled with a sanguinous exudate. Rats in 10 percent carbon dioxide survived for the duration of the experiment. Stephens (43) exposed rats to 20 percent carbon dioxide for 2 hours and then to gradually increasing concentrations up to 43 percent. The oxygen content was maintained at 18 to 21 percent throughout the experiment. Survival times were 2.5 to 19.3 hours. Damage to the brain and spinal cord was severe and in direct proportion to the animals' exposure. The greatest changes in the central nervous system were in the optic thalamus, brain stem, and spinal cord. Swelling of
nerve cells was evident with vacuolization and shrinkage of nuclei, marked chromatolysis, swollen oligodendroglia, and pyknotic neuroglial nuclei.

Accidental deaths from exposure to extremely high levels of carbon dioxide have been reported. Troisi (44) reported the collapse and death of three men after descending into a silo. Two died at the scene and the third, 5 days later. The clinical picture was that of asphyxia. It has been stated that the carbon dioxide concentration in silos can reach 38 percent (45). Although Troisi (44) attributed the deaths to carbon dioxide exposure, no analyses were reported. Since nitrogen oxides are also produced during ensilage, their contribution to the lethal atmosphere cannot be ignored. Deaths from carbon dioxide have also been reported in holds of ships (46,47), deep artesian wells (48,49), and mines (50). Poisoning attributed to carbon dioxide has also been reported among firefighters (51) and aircrew members (52), although atmospheric concentrations were not reported.

**Short-term studies**

Schaefer et al. (53) exposed guinea pigs to 15 percent carbon dioxide in 21 percent oxygen for 7 days. The animals lost weight during the first 2 days but returned to their original weight before their removal from the exposure chamber. The blood corticosteroids rose markedly and adrenal epinephrine fell during the first 3 days of exposure. During this period, there was also a rise in arterial free fatty acids and a decrease in lymphocytes and adrenal cholesterol. These changes suggest a stimulation of the sympathetic system by chronic hypercapnia.

Barbour and Seevers (42) noted a weight loss of 14 to 27 percent in rats exposed to 10 percent carbon dioxide for 30 days and of 50 percent when exposed to 20 to 25 percent carbon dioxide for 34 days. They attributed this loss to reduced food intake, for the rats ate sparingly at carbon dioxide concentrations above 10 percent. When returned to normal atmosphere, the rats regained the lost weight within a few days.

Stein et al. (54) placed 10 healthy male rhesus monkeys in a sealed chamber containing 3 percent carbon dioxide and 21 percent oxygen. One monkey died of shigellosis on the 62nd day. The other nine animals remained in the chamber for 93 days and displayed no significant difference from controls in any of the parameters measured: dietary habits, weights, respiratory rates, hematologic indices, water intake, blood chemistries, and general activity. Necropsies were performed on five of the animals at the end of the experiment and on the other four from 28 to 46 days later. No abnormalities were detected.
Long-term studies

No reports of long-term studies were found by the Select Committee.

Teratogenesis and effects on the reproductive system

The fertilizing capacity of avian spermatozoa was totally destroyed when semen were stored under carbon dioxide at 21 atmospheres for 7 hours at 11°C (55). Male Swiss mice were subjected to 35 percent carbon dioxide for periods of 1 to 2 hours for a total exposure of 6 hours (56). The width and area of the sperm head and midpiece were significantly less than sperm from control mice. Normal female mice, mated to males exposed for a total of 0.5 hours to 35 percent carbon dioxide over a period of 6 days had a reduced conception rate but normal litter size. VanDemark et al. (57) exposed mature male Wistar rats to 2.5, 5.0, or 10 percent carbon dioxide for 1, 2, 4, or 8 hours, and killed them immediately after exposure. Degenerative changes in the seminiferous tubules were observed that roughly paralleled the carbon dioxide concentration and duration of exposure.

Haring (58) exposed pregnant Sprague-Dawley rats to 6 percent carbon dioxide for single 24-hour periods between days 5 and 21 of pregnancy. Cardiac abnormalities were reported in 23.4 percent of the offspring of test animals and included high and low interventricular septal defects, overriding aortae, partial transposition, and pulmonic or aortic stenosis. Among controls, the only malformation observed was a persistent interventricular foramen in 6.8 percent of the animals. The greatest incidence of malformations occurred when the dams were exposed to carbon dioxide on the tenth day of pregnancy. An increased incidence of skeletal malformations (kyphosis or hydrocephalus) was also observed: 10.9 percent in the test and 0.6 percent in the control group.

Possible teratogenic effects have also been reported in rabbits (59). Three rabbits were exposed to 10 to 13 percent carbon dioxide for 4 to 10 hours on 2 or 3 days between days 7 and 12 of pregnancy. Of 67 pups from 11 litters, 16 had congenital malformations in the cervical, thoracic, or lumbar vertebral column. The malformations among the males were three times those of the females. Of 30 control progeny, only one malformation was seen.

Forty guinea pigs were exposed to 0.48 percent carbon dioxide for 10 minutes daily for 20 days beginning on day 20 of pregnancy (60). Twenty-seven of the dams miscarried. Of the pups carried to term, 18 were normal but 5 showed varying degrees of flaccid paralysis of the hind limbs. A second group of 40 guinea pigs was exposed to 0.42 percent carbon dioxide for 1 hour daily for 30 days, also beginning on day 20 of pregnancy. All litters
were carried to term but 39 (of 85) of the offspring were microsomic and 24 of these had neuromuscular defects of the hind limbs. No skeletal malformations were seen. All control litters were normal.

Carcinogenesis

Solid carbon dioxide (dry ice) used as a "chronic irritant" was reported in 1931 to produce papillomas and carcinomas in mice. The dry ice was applied daily to the backs of mice, momentarily freezing the skin site. Of 30 animals, 26 developed papillomas and 2 developed carcinomas. After 390 days, 6 of the 30 mice were still alive (61).

Mutagenesis

No reports on possible mutagenic effects of carbon dioxide were available to the Select Committee.

Special studies

Ambient carbon dioxide concentrations in a brewery were monitored constantly for 5 days (62). The time-weighted-average concentrations varied from 0.5 to 1.95 percent with a mean of 1.08 percent; momentary concentrations reached 8 percent. After studying the workers in the various brewery sites, the investigators concluded that there was no significant physiological effect of chronic intermittent exposures to these levels of carbon dioxide.
V. OPINION

The Select Committee could find no data relating directly to the safety of carbon dioxide as a food ingredient. However, there is substantial evidence that the amount of carbon dioxide ingested with foods is negligible compared with that produced normally by the body. Carbon dioxide also has been administered experimentally without ill effects in amounts orders of magnitude greater than from possible food sources.

Under resting conditions, an average adult will produce in excess of 500 g of carbon dioxide daily, approximately 2000 times the estimated intake from foods. During moderate or heavy activity, considerably greater amounts of carbon dioxide would be produced. The official occupational standard for the workroom atmosphere is 5000 ppm, equivalent to the inhalation during an 8-hour work day of about 30 g of carbon dioxide. Human subjects have been exposed to 1.5 percent carbon dioxide (a daily inhalation of over 200 g) continually for 42 days without serious or lasting ill effects.

The amount of bicarbonate that could be produced from the ingested carbon dioxide is far less than the amounts routinely ingested in food or used without ill effects as an antacid.

The Select Committee is not aware of any study on the mutagenicity of carbon dioxide. Carcinogenic actions have been reported only after repeated application of the solid form to the skin. Teratogenic effects were produced after inhalation of carbon dioxide at levels far higher than could be obtained from foods.

In consideration of these factors, the Select Committee concludes that:

There is no evidence in the available information on carbon dioxide that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

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