EVALUATION OF THE HEALTH ASPECTS OF STARTER
DISTILLATE AND DIACETYL AS FOOD INGREDIENTS

1980

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 Administration (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 assessments 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 Administration, after announcement in the Federal Register, and opportunity 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 conclusions. 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 Societies 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 starter distillate and diacetyl as food ingredients. 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; recent literature searches by the Toxicology Information Response Center, Oak Ridge, Tennessee; 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, announcement was made in the Federal Register of January 12, 1979 (44 FR 2687) 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 using starter distillate and diacetyl as food ingredients or, in lieu of an oral presentation to submit a written statement. A hearing was held on July 16, 1979, in response to a request for opportunity to make an oral presentation. Information on the hearing is given on page 22.

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 Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the

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*The document (PB-234 896) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
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 starter distillate and diacetyl and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

Starter distillate, or butter starter distillate, is a flavoring used to impart a butter-like flavor to processed foods (3-5). Manufacturers sell it to the food industry under the name starter distillate. Producers of such products as butter prefer to identify the substance as butter starter distillate. The Food and Drug Administration (6) regards starter distillate and butter starter distillate as different names for the same product.

Starter distillate is a steam distillate of the culture of any or all of the following species of bacteria grown on a medium consisting of skim milk usually fortified with about 0.1 percent citric acid: Streptococcus lactis, S. cremoris, S. lactis subsp. diacetylactis, Leuconostoc citrovorum, and L. dextranicum (7-14). Water comprises more than 98 percent of the weight of the distillate; the remainder is composed principally of a mixture of flavor compounds (12,14). Starter distillate is used as a flavor enhancer, principally in margarine but also in lesser quantities in a variety of other products.

Of the many flavor compounds identified in starter distillate, diacetyl is the major component, comprising as much as 80 to 90 percent of the mixture of flavor compounds (1,6,8,14-17). Ferric chloride is added to the distillation pot during production of starter distillate to increase the yield of diacetyl, due to the oxidation of acetoin with ferric chloride (15,18). Commercial starter distillates are commonly standardized on basis of diacetyl concentration at levels of 1.0 mg per ml and 15 mg per ml. The latter level is obtained by segregating the early distillation cuts and blending them to give the desired level.

\[ \text{Diacetyl, CH}_3-C-C-\text{CH}_3, \] is also known as 2,3-butanedione, dimethyldiketone, dimethylglyoxal, biacetyl, and 2,3-diketobutane. It is synthesized chemically from methyl ethyl ketone (19). The Food Chemicals Codex (20) specifies the product should contain not less than 97 percent diacetyl, not more than 3 parts per million arsenic, 59 ppm heavy metals measured as lead and 10 ppm lead. It is a clear yellow to yellowish green liquid with a strong pungent odor. In very dilute solution, it has a typical buttery odor and flavor. It is miscible in glycerin and in water, but is insoluble in mineral oil. No specifications for starter distillate are given in the Food Chemicals Codex (20).

Starter distillates from the two domestic manufacturers of these products were analyzed by Lindsay (14) employing gas chromatography (GC) and mass spectrometry. Table I presents a list of the compounds identified in ether extracts of starter distillates and estimates of their concentrations. Acetaldehyde, ethyl formate, ethyl acetate/acetone (unresolved GC peak), ethanol/butanone
<table>
<thead>
<tr>
<th>Overall designated peak number*</th>
<th>Compound</th>
<th>Concentration in starter distillate**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Acetaldehyde</td>
<td>less than 500 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl formate</td>
<td>less than 500 ppm</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate/acetone</td>
<td>less than 1000 ppm</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol/2-butanone</td>
<td>less than 500 ppm</td>
</tr>
<tr>
<td>7</td>
<td>Diacetyl</td>
<td>1000-15000 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Ethyl butyrate</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Butyal acetate (tentative)</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>10</td>
<td>Butanol (tentative)</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>13</td>
<td>Ethyl hexanoate</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>14</td>
<td>Acetoin</td>
<td>less than 50 ppm</td>
</tr>
<tr>
<td>18</td>
<td>Acetic acid</td>
<td>250-3000 ppm</td>
</tr>
<tr>
<td>19</td>
<td>Ethyl octanoate/2,3-butanediol</td>
<td>less than 5 ppm</td>
</tr>
<tr>
<td>20</td>
<td>Furfural</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>22</td>
<td>5-methyl-2-furfural (tentative)</td>
<td>less than 10 ppm</td>
</tr>
<tr>
<td>24</td>
<td>Furfurol</td>
<td>less than 10 ppm</td>
</tr>
</tbody>
</table>

*Peaks listed represent the major peaks; other small peaks are due to browning intermediates (furans, maltol, acetol, etc.), but each represents less than 5-10 ppm of the starter distillate (14).

**Based on assumption of equivalent gas chromatographic detector response and extraction recoveries.
(unresolved GC peak), diacetyl, and acetic acid represent the major organic components. All other components except acetoin were present in concentrations less than 10 ppm. The values listed for diacetyl do not represent limiting values of a range of concentrations but are the two standard concentrations present in commercial products.

Average and range of concentrations of the major components (other than diacetyl and acetic acid) found in six samples of commercial starter distillates having a diacetyl concentration of 15 mg per ml are given in Table II. Lindsay (14) stated that concentrations of these components in starter distillates standardized to 1 mg diacetyl per ml (7 samples from two manufacturers examined) generally fall at the lower end of the range given in Table II.

Analysis of starter distillates by a gas chromatographic method for free fatty acids showed that C₄ through C₁₈ fatty acids were present at levels below 5 ppm (14). Volatile fatty acids were intentionally excluded from the ether extracts to avoid their interference with other components; acetic acid was determined by direct injection gas chromatographic analysis. Only slight indications of formic acid were indicated by the latter method.

Based on the concentrations of organic components given in Tables I and II, and assuming there may be as many as 50 components present at concentrations of 10 ppm, the combined concentration of organic components other than diacetyl and acetic acid is estimated to be less than 2.4 mg per ml (about 0.2 percent) in a starter distillate having a diacetyl concentration of 15 mg per ml. In starter distillates having a diacetyl concentration of 1 mg per ml, the concentration of organic compounds other than diacetyl and acetic acid, is similarly estimated to be less than 1 mg per ml (about 0.1 percent).

In an earlier study, Lindsay et al. (8) identified acetaldehyde, ethyl formate, ethyl acetate, butanone, ethyl octanoate, 2-furfural, and 2-furfurol as components of the heated milk used as the culture medium for the production of starter distillate. Evaluation of the health aspects of acetic acid (21), and formic acid and ethyl formate (22) has been presented in other reports of the Select Committee.

While no published specifications for starter distillate have come to the attention of the Select Committee, one manufacturer has provided a general description which states that starter distillate is a mixture of flavor compounds distilled from specially cultured skim milk, the main flavor component of which is diacetyl. It is used in the manufacture of butter, margarine, cottage cheese, sour cream, and candies. The manufacturer suggests that for prod-
TABLE II

Concentrations (mg per ml) of the Most Volatile Compounds in Ether Extracts of Commercial Starter Distillates Having a Diacetyl Concentration of 15 mg per ml (14)

<table>
<thead>
<tr>
<th></th>
<th>Acetaldehyde</th>
<th>Ethyl formate</th>
<th>Ethyl acetate/acetone</th>
<th>Ethanol/2-butanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.25</td>
<td>0.37</td>
<td>0.42</td>
<td>0.16</td>
</tr>
<tr>
<td>Range</td>
<td>0.07–0.50</td>
<td>0.06–0.49</td>
<td>0.12–0.73</td>
<td>0.06–0.34</td>
</tr>
</tbody>
</table>
TABLE III

Level of Addition of Butter Starter Distillate, Diacetyl, and Starter Distillate to Foods by Food Category (3)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Butter starter distillate*</th>
<th>Diacetyl weighted mean percent</th>
<th>Starter distillate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>0.030</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Fats and oils</td>
<td>0.111</td>
<td>0.001</td>
<td>0.070</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>Cheese</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>0.044</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft candy</td>
<td>0.034</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>0.008</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Snack foods</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Hard candy</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chewing gum</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Blanks in the table mean that the substance is not added to the foods indicated. Level of addition of butter starter distillate, diacetyl, and starter distillate is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean, see text, also Section X and Exhibit 50 of reference 3. Butter starter distillate and diacetyl use was volunteered information by some of the industries surveyed.

*Butter starter distillate and starter distillate are considered to be identical substances by the Food and Drug Administration.
ucts like cottage cheese and margarine it is desirable to have about 1 to 5 ppm in the final products (12). Riel and Gibson (23) have discussed its use in flavoring butter at various concentrations.

In an effort to determine organoleptic preferences for levels of flavor constituents in dairy products, Lindsay et al. (16) prepared formulations with various concentrations of flavor substances identified as present in dairy products and presented them to a panel for organoleptic evaluation. Culture flavor formulations considered best by their panel provided 0.2, 0.2, and 0.5 mg acetaldehyde per kg of sour cream, cottage cheese, and buttermilk, respectively, and either 1 or 2 mg diacetyl per kg product. Further, with regard to levels of usage of starter distillate in foods, Lindsay (15) has stated that the maximum levels of usage of starter distillate in foods will be limited by the disagreeable flavor of the diacetyl. Diacetyl exhibits a desirable flavor over a range of 0.05 ppm to about 5.0 ppm, but above this it becomes quite obnoxious and disagreeable. Similar conclusions were reached by Hempenius et al. (24) in studies of consumer preference for flavor in creamed cottage cheese.

Starter distillate is presumed to be GRAS by the Food and Drug Administration but not published (3). It is also approved under Title 9, Sec. 318.7 of the Code of Federal Regulations "to help protect the flavor" of oleomargarine (25). Butter starter distillate is listed in the Code under 21 CFR 172.505 as a synthetic flavoring substance or adjuvant that may be used in accordance with good manufacturing practice (2). Diacetyl is listed in the Code under 21 CFR 182.60 as a synthetic flavoring substance or adjuvant generally recognized as safe (GRAS) for its intended use. It is also approved as a flavoring agent in amount sufficient for the purpose in the manufacture of margarine (25).

In addition to diacetyl, the following substances reported by Lindsay (14) as components of starter distillate are listed in the following sections of the Code of Federal Regulations (2,25):

Acetaldehyde, acetoin, ethyl butyrate, and ethyl acetate are generally recognized as safe as synthetic flavoring substances and adjuvants (21 CFR 182.60); ethanol is affirmed as GRAS as an antimicrobial agent (21 CFR 184.1293); acetic acid as a GRAS substance migrating from cotton and cotton fabrics used in dry food packaging (21 CFR 182.70), as a substance migrating to food from paper and paperboard products (21 CFR 182.90), as a multiple purpose GRAS food substance (21 CFR 182.1005), and as a refining agent of rendered fats to separate fatty acids and glycerol (9 CFR 318.7).
The following substances also identified as components in starter distillate, are listed in the Code (2) under 21 CFR 172.515 as synthetic flavoring substances and adjuvants:

- 2-butane
- 1-butanol
- butyl acetate
- ethyl formate
- ethyl hexanoate
- ethyl octanoate
- formic acid

In 1968 the FAO/WHO Expert Committee on Food Additives (26) listed diacetyl among compounds for which exposure recommendations could not be made because of lack of data, but stated that tentative specifications were available on request. It concluded that no distinction should be made between a substance occurring naturally and the same compound made synthetically. The Committee limited its comments on diacetyl to purity of the substance and its physical constants, i.e., to assay not less than 97 percent, refractive index $N_D^2$ 1.3930-1.3970, specific gravity 0.975-0.990, and solidification point -1.0° to -4.0° (27).
III. CONSUMER EXPOSURE DATA

A National Research Council (NRC) subcommittee (3) has provided data on the mean level of addition of starter distillate, butter starter distillate, and diacetyl to foods (Table III).

According to data collected by a NRC subcommittee (3), 18,000 kg of diacetyl was utilized as a food ingredient in 1970. Industry estimates of starter distillate (equivalent to product standardized at 1 mg diacetyl per ml) sales to the food industry in 1978 were 300,000-400,000 kg (18). Thus, the consumption of aqueous starter distillates may be estimated to be about 5 mg per capita daily. Since total concentration of organic components other than diacetyl and acetic acid was estimated to be less than 0.1 percent (see p. 3) in starter distillate standardized at 1 mg diacetyl per ml, daily per capita intake of these components would be less than 5 µg or 0.08 µg per kg body weight.

Based on the 18,000 kg of diacetyl reported to be added to foods as such, and the content of diacetyl in the starter distillate used as a food ingredient, it is estimated that daily consumption of diacetyl from these sources is less than 0.3 mg per capita. Although diacetyl occurs naturally in a wide range of foods (28), no quantitative estimates were available to the Select Committee on the exposure of diacetyl from these sources.
IV. BIOLOGICAL STUDIES

Absorption and metabolism

No in vivo studies of the absorption and metabolism of starter distillate or diacetyl were available to the Select Committee. However, certain in vitro studies of diacetyl are relevant.

In an investigation of the metabolism of diacetyl by rat liver slices and homogenates under aerobic conditions, Järnefelt (29) found that homogenates (few intact cells) converted part of the diacetyl to acetoin (acetylcarbinol) but did not metabolize the acetoin. However, liver slices (intact cells) converted all of the diacetyl to acetoin and further metabolized the acetoin.

Utilizing Pseudomonas species for studying the glyoxylate cycle, Hullin and Hassall (30) grew cultures in which butane-2,3-diol was used as the sole source of carbon. Extracts of these cells catalyzed the conversion of diacetyl to 3-hydroxy-3-methyl pentane-2,4-dione. Isocitratase activity of the cell extract was as high as when acetate was used as the carbon source and 20-fold higher than when succinate was used. In the presence of acetate, diacetyl, 2-hydroxybutane-3-one and cofactors, the cells transferred labeled carbon from glyoxylate to malate, which showed that diacetyl could be metabolized through the glyoxalate cycle.

Using a "rat liver mince" to verify in mammalian systems the enzymatic pathways found in bacteria for the interconversion of diacetyl, acetoin, and 2,3-butanediol, Gabriel et al. (31) incubated the mince with 1000-fold higher than physiological concentrations of acetoin. The acetoin disappeared coincident with the formation of 2,3-butanediol but with very little elaboration of carbon dioxide. They isolated two enzymes: acetoin dehydrogenase (EC 1.1.1.5) and 2,3-butanediol dehydrogenase (EC 1.1.1.4) that were nicotinamide adenine dinucleotide positive-dependent or nicotinamide adenine dinucleotide phosphate positive-dependent, and differed from alcohol dehydrogenase. The activity of the latter enzyme diminished on dialysis against disodium ethylene-diaminetetraacetate and was restored by addition of Co++, Cu++, Zn++, and other divalent metal ions. Chromatography and electrophoresis showed rat liver diacetyl reductase to consist of multiple proteins.

Another pathway for diacetyl metabolism was reported by Shinagawa et al. (32) who found citrate to be formed from diacetyl and oxaloacetate in the presence of diphosphothiamin and acetyl CoA, with negligible oxygen consumption in dog heart homogenates. The conversion was unaffected by malonate, diphosphopyridine nucleotide, or adenosine triphosphate.
Acute toxicity

The Registry of Toxic Effects of Chemical Substances (33) makes no mention of starter distillate, and no acute toxicity studies were found elsewhere by the Select Committee. However, LD<sub>50</sub> data for several of the reported constituents of starter distillate are given in Table IV. Acute toxicity data for diacetyl are given in Table V.

Short-term studies

Groups of 15 male and 15 female SPF-derived CFE rats housed five per cage were fed daily by intubation, 5 ml per kg of a solution of water containing zero, 0.2, 0.6, 1.8 or 10.8 percent of diacetyl (0, 30, 90 or 540 mg per kg) for a period of 90 days (36). Water and food were given ad libitum. All of the rats were killed and necropsied at 90 days. During the in vivo period of the study, there were no significant differences between any of these groups in blood analyses, urine, and liver and kidney function. However, growth rate was less and water consumption was greater in the highest dose group (540 mg per kg), especially for the males.

Rats that received 540 mg diacetyl per kg per day had lower hemoglobin values, smaller packed cell volume, and higher reticulocyte and leucocyte counts (mainly neutrophils) than the others; there were no sex differences (36). The absolute organ weights in males at the highest treatment level were 5 to 15 percent less than those of control animals; final body weights were 25 percent less than those of controls. Thus, organ to body weight ratios were greater than those of the controls. An exception to this was in the adrenal glands; both absolute and relative weights were greater than those of the controls. In females, the absolute and relative weights of liver, kidneys and adrenals of rats in the highest dosage group were greater than those of control animals; relative weight of the pituitary was greater in the treated female rats. Gastric squamous epithelium in these animals sloughed, and ulcers were seen microscopically. This could explain the anemia, leucocytosis and differences in organ weights. The authors stated that the greatest no-adverse-effect level of diacetyl given orally to rats was 90 mg per kg per day. They estimated that human intakes in the United Kingdom would approximate 10 mg per day per person, and concluded that the calculated human maximum intakes on a body-weight basis (0.17 mg per kg) would be approximately 1/500 the no-effect (90 mg per kg) level for rats.

No short-term studies of starter distillate or long-term studies of either starter distillate or diacetyl were available to the Select Committee.
TABLE IV

Acute Oral Toxicities in the Rat of Several Constituents of Starter Distillate

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD$_{50}$ mg/kg</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>1930</td>
<td>34</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3310-3530</td>
<td>34</td>
</tr>
<tr>
<td>Acetone</td>
<td>9750</td>
<td>34</td>
</tr>
<tr>
<td>2-butanone</td>
<td>3400</td>
<td>33</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>4130</td>
<td>34</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>5620</td>
<td>34</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>13050</td>
<td>34</td>
</tr>
<tr>
<td>Ethyl formate</td>
<td>1850</td>
<td>34</td>
</tr>
<tr>
<td>Ethanol</td>
<td>13660</td>
<td>34</td>
</tr>
<tr>
<td>2-furfural</td>
<td>127</td>
<td>33</td>
</tr>
<tr>
<td>Furfurol</td>
<td>275</td>
<td>33</td>
</tr>
</tbody>
</table>

TABLE V

Acute Toxicity of Diacetyl After 18-Hour Fast

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>LD$_{50}$ mg/kg</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>p.o.</td>
<td>1580</td>
<td>35</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>p.o.</td>
<td>990</td>
<td>35</td>
</tr>
<tr>
<td>Rat/M</td>
<td>p.o.</td>
<td>3400</td>
<td>36</td>
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<td>Rat/F</td>
<td>p.o.</td>
<td>3000</td>
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<td>Rat/M</td>
<td>i.p.</td>
<td>400</td>
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<tr>
<td>Rat/F</td>
<td>i.p.</td>
<td>640</td>
<td>36</td>
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Special studies

Mutagenicity studies of diacetyl available to the Select Committee are limited to those of Loveless (37) who found that this compound had no genetic activity on root-tip meristems of Vicia faba. In another study by Barthelmess and Elkabarity (38) a number of substances were tested for influence on genetic expression using root meristems; diacetyl diluted to 0.08-0.10 mol per liter was found to delay metaphase and consequently to increase the frequency of observed anaphase.

Mutagenic evaluation of starter distillate tested with Saccharomyces cerevisiae (strain D4), and Salmonella typhimurium strains activated with tissue homogenates and supernatants from liver, lung and testis of mouse, rat and primate did not reveal mutagenic effects (39).

In teratogenicity studies, oral intubation of up to 1600 mg per kg body weight of starter distillate to pregnant mice and rats daily from day 6 through day 16 of gestation, to pregnant hamsters daily from day 6 through day 10 of gestation, and to pregnant rabbits daily from day 6 through day 18 of gestation, demonstrated no evident effect on nidation or on maternal or fetal survivals (40). The number of abnormalities of soft or skeletal tissues did not differ from the number occurring spontaneously in sham-treated control animals.

Carcinogenicity

The Select Committee is not aware of any studies on the carcinogenicity of diacetyl or starter distillate. However, Arcos et al. (41,42) have used diacetyl as one of a group of carbonyl compounds known to interact with sulfhydryl groups of proteins in studies of inhibition of the carcinogenic activity of aminoazo dyes such as 3’-methyl-p-dimethylaminoazobenzene.
V. OPINION

Diacetyl is added to some foods for flavoring purposes. It is metabolized in mammals, is of low acute toxicity, and the no-adverse-effect level, based on a 90-day study in rats, is approximately 90 mg per kg body weight. The per capita daily intake of diacetyl added to food, both as a component of starter distillate and as diacetyl itself, is estimated to be less than 0.3 mg.

Available studies of the biological effects of commercial starter distillate consist of two recent reports; the one showed that starter distillate exhibited no mutagenic activity in in vitro test systems; the other showed that it was without teratogenic activity when administered orally in doses as high as 1600 mg per kg body weight to pregnant mice, rats, hamsters, and rabbits.

The per capita daily intake of starter distillate is about 5 mg or about 0.1 mg on an anhydrous basis. Diacetyl and acetic acid are major components of starter distillate; total daily per capita intake of all organic components of starter distillate, other than diacetyl and acetic acid, is estimated to be less than 0.08 μg per kg body weight. Based on the nature of the starting material and the process used to produce starter distillate, the Select Committee has no grounds to suspect that the small amount of unidentified ingredients poses a hazard. It would appear that the possibility of hazard from the addition of starter distillate is minimal. However, no food grade standards exist for starter distillate. It is a mixture of many substances, not all of which have been identified, whose qualitative and quantitative composition may vary depending on the combinations of microorganisms used in the starter culture, and on the conditions of steam distillation. Hence, there is need for establishing practical food grade standards for starter distillate specifying acceptable limits of variability.

In light of these considerations the Select Committee concludes that:

There is no evidence in the available information on diacetyl or starter distillate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

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VI. REFERENCES CITED


17. Memorandum of telephone conversation, August 2, 1977, between Mr. Beery (plant manager), Chumlea's Laboratories, Inc., Lebanon, Ind., and A.F. Freeman, Federation of American Societies for Experimental Biology, Bethesda, Md.


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Report submitted by:

March 21, 1980

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Select Committee on CNAS Substances
PUBLIC HEARING ON STARTER DISTILLATE AND DIACETYL
HELD ON JULY 16, 1979

A statement was presented by Neil Dinesen, Chr. Hansen's Laboratory, Inc., Milwaukee, Wis. No other statements were presented at the hearing.
