The Chick Edema Factor and Its Relation to the Fatty Acid Industry[1]

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When the 85th Congress in September, 1958 passed and sent to the President for his signature an amendment to the Federal Food, Drug, and Cosmetic Act regulating chemical additives to food, hardly an eyebrow was lifted among fatty acid producers because of the long historical recognition of these materials as safe.

This feeling of security was further enchanced with the issuance of the tentative GRAS (Generally Recognized As Safe) list in the Federal Register on December 9, 1958. Oleic and linoleic acids, glyceryl monostearate, magnesium stearate and mono and diglycerides, except lauric, were listed. As stearic acid is a component of glyceryl monostearate and magnesium stearate, and is often found in mono- and diglycerides, it was felt that stearic acid was included by implication and the failure to list it separately was simply an oversight.

This was found to be the case when a query was made to the Food and Drug Administration, for the reply received March 16, 1959 by Fatty Acid Producers Council said in part: "We concur in the opinion that stearic acid is generally recognized by appropriately qualified experts as being safe for food use and that it should not be subject to clearance provisions of the Food Additive Amendment for those uses which are customary and usual."

Here things stood until a reading of the revised GRAS list issued November 20, 1959, indicated that instead of correcting the stearic acid oversight, now oleic and linoleic acids were also omitted although, strangely, glyceryl monostearate remained. A revision of the section dealing with emulsifying agents listed a group of mono- and diglycerides requiring their production from edible fats and oils by glycerolysis. The glycerolysis limitation was the only processing requirement shown by the entire GRAS list and the raw material source statement "of edible fats or oils," also was the only such requirement on the list.

The Fatty Acid Council speedily appointed a committee to meet with the FDA to establish the background for these changes. It didn't take long to find out that the FDA was now concerned about U.S.P. Oleic Acid because of certain samples they had picked up in a plant manufacturing esters for food use and found them to affect chicks with symptoms indicating a similarity to the chick edema disease of 1957 (1,2,3,4,5,6,9). That outbreak had been traced to a fatty acid still residue product high in unsaponifiables and this new finding relative to low unsapouifiable material was unexpected. These oleic acids were found to abnormally affect rats and monkeys, as did a food grade glyceryl monooleate having 0.22% unsaponifiable, while a glyceryl trioleate was even more active. This finding was also reported later by Dr. Ames and his group at Distillation Products Industries, who had this effect called to their attention by F. W. Hill of Cornell University. He had observed abnormal effects with a food grade glyceryl monooleate.

The group at Distillation Products Industries found that conversion of these oleics to glyceryl esters carried over the C.E.F. (chick edema factor) in proportion to its presence in the fatty acids. Hence, the attempt of the FDA to prevent this possibility in the production of monoglycerides by spelling out the "glycerolysis" limitation. It was felt by the FDA, based on the evidence at the time, that the C.E.F. was somehow related to fatty acid distillation procedures.

Cooperative work between the FDA, some association members and feed manufacturers trying to track down the cause of the 1957 edema outbreak, indicated that split fatty acid stock was satisfactory but distillation of the split acids showed some C.E.F. in the distillates, with even more in the still residues. Hence the working theory at the time was that distillation somehow caused the appearance of the C.E.F. One group in the FDA felt the effect was thermal in origin, while others felt it might be contamination from something such as boiler treating compounds used in the production of the sparging steam for fatty acid distillation.

It was because of the association of the 1957 edema outbreak with low grade inedible tallows that the FDA now spelled out the edible requirement for monoglycerides even though section 402 of the act covered this matter.

While no evidence had appeared that stearic acid had the C.E.F., it was omitted from the GRAS classification because of the possibility that it could be produced from oleic acid by low pressure hydrogenation, which was known not to affect the C.E.F. (4). In other words, the responsibility was placed on the manufacturers to define a grade of stearic acid that was safe for food use, rather than to exempt the commerical product generally under the GRAS clause.

This situation caused considerable concern among fatty acid and emulsifier producers because it appeared to strike at the root of two of their key processes, namely, distillation and esterification. The concern was accentuated by the short time interval allowed to get in under the March 6, 1960 enforcement deadline by petitioning for a time extension.

The fatty acid industry set in motion a very intensive search for chick edema positive materials. Screening tests utilizing hydrocarbon diene content, a cholestadiene test, and chick feeding tests, were tried. Cooperative work with samples of C.E.F. positive commercial oleic acids kindly furnished by D.P.I. rapidly established the worthlessness of the cholestadiene and hydrocarbon diene tests, leaving only chick feeding tests to fall back on, which incidentally is where we stand today.

Steps were also taken to set in motion the requirements for obtaining a time extension of the enforcement.

In the spring of 1960 while the fatty acid industry was searching its supplies and processes to find C.E.F. positive material, the Food and Drug inspectors picked up quantities of both acids and derivatives in the field, from various producers, for their own evaluation.

The upshot of all this activity was that everyone came up with negative results. No C.E.F. appeared anywhere, not even in first, second, or third still bottoms produced from oleic or cottonseed acids, much less in the distillates, or in stearic acids. This threw cold water on the heat and distillation theories for the C.E.F. formation.

At about this time, the Association became acquainted with the concentration and isolation work on the C.E.F. being done by a group of workers at Merck & Co. and Procter & Gamble. Merck had assigned the men who worked on the isolation of vitamins B_6 and B_{12} to this task, and, in conjunction with a quantitative bioassay procedure developed by Dr. Ott of their nutrition labs, were following the isolation step by step from large quantities of the tallow held over from the 1957 outbreak (12).

Dr. Norman Brink and his group at Merck shortly reported the isolation of what they felt was the purified C.E.F. They had obtained 1 mg from approximately 1000 lb. of starting edema positive tallow (11). Help was asked of the Association members in supplying more concentrated sources of such edema positive fat for further work.

Shortly thereafter, Merck definitely found halogen in the pure C.E.F., due to work by Dr. Trenner and his group. Within a few more weeks it was established that the halogen was chlorine, present to the extent of about 47% in a nonaromatic molecule, having from 10–12 carbon atoms, but showing evidence of a diene structure. It sublimed at about 225°C but it was felt that this figure might be as high as

[1] Presented at the 35th Fall Meeting of the American Oil Chemists' Society.

260°C. A molecular weight of approximately 380 was indicated. This agreed well with the figure previously reported by Dr. Ames based on molecular distillation work (7). The high biological activity of 1.6 million times that found in the crude fat, which readily killed chickens, rated this as a potent toxin. In the chick diet, a level of 16 parts per billion showed a positive response.

With the first finding of chlorine, and before the molecular level was established, the possibility that chlorine dioxide bleaching of fats might be the cause of the C.E.F. was checked by submitting some tallow, treated in this manner, and also after prolonged heating subsequent to bleaching to Dr. Ott, who found these to be negative by his bioassay procedure. The positive finding of a highly chlorinated molecule, in the pure C.E.F., was also confirmed by workers at Procter & Gamble and the FDA, who separately isolated this factor about this time. A comparison of the infrared curves obtained by Merck and the FDA showed structural differences, and among these was the indication of aromaticity in the FDA sample. Dr. Artman reported the isolation work of the C.E.F. done by Procter and Gamble at the 1961 A.O.A.C. convention.

As a result of these findings, the Merck workers were convinced that they were dealing with an unnatural material resulting from contamination and decided to discontinue any further work. Before doing so, they tested a number of chlorinated cyclodiene pesticides, as did a number of the members of our Association, the FDA, and Procter & Gamble. Also checked were chlorinated naphthalenes which had been implicated in toxic effects on cattle some years before. The workers at the FDA leaned toward the chlorinated naphthalene theory for the C.E.F., based on their data. The pesticide theory for the C.E.F. seemed the most plausible interpretation of the Merck results. To check these possibilities, several pesticides and lubricants were tested and, although found toxic to varying degrees to chicks, in no case did they show positive edema results. They were: Aldrin, Dieldrin, Endrin, DDT, Chlordane, Hepthachlor, Hexachlorophene, tetrachlorotetrahydronaphthalene, hexachlorocyclohexane, pentachlorophenol, tetrachlorotoluene and hexachloronaphthalene.

The Association also sponsored small scale rendering tests at the American Meat Institute using tallow having Aldrin, Dieldrin, Endrin and Heptachlor added at a level designed to give 10 p.p.m. of contaminant in the rendered fat. These were all found to be negative to the chick feeding test. Fly tests on the rendered fats showed 100% mortality in each case, indicating the presence of the insecticides. Prior fly tests by the same company on known CEF positive tallow and oleic acids had shown no insecticidal activity.

Another company ran the gamut of available insecticides in testing for thermal breakdown products, by heating them at a 1% level in fatty acids to over 200°C for extended periods of time. In no case did a positive edema test result on feeding the acids to chicks.

While this work was proceeding, the FDA issued a time extension covering food grade stearic, oleic, and related straight chain acids, and also the mono- and diglycerides from them, free of the chick edema factor and from edible fats and oils. The glycerolysis limitation had been dropped. The ice cream standards issuing a few months later had this limitation, which the commissioner later rectified, on his own initiative, after we pointed out the discrepancy.

In September of 1960 another outbreak identified by a number of independent laboratories as "Chick Edema Disease" occurred in some brooder houses in Georgia. The C.E.F. positive feed concentrate was traced to the production of a single day. Unfortunately, neither the fat supplier nor the feed manufacturer retained fat samples, so it has not been possible unequivocally to prove that contaminated fat, which in this case was No. 1 tallow, was the cause. The various other feed components were checked by feeding and found not at fault.

Our laboratory received samples of the C.E.F. positive feed and extracted the fat. This was tested for chlorinated material by a member laboratory using a Dohrman instrument and also using the Mills pesticide procedure. A finding of approximately 50 p.p.m. of Chlordane was noted. That this fat contained the C.E.F. was established by feeding tests indicating a moderate level of toxicity. Other

nontoxic feeds taken from production about the same time showed a few p.p.m. of Chlordane, BHC and Lindane and were found nontoxic on checking the extracted fat. The fat supplier was positive that the particular shipment of No. 1 tallow contained no fatty acid residues of any kind.

Approximately 12,000,000 chickens were fed this toxic supplement utilizing 4000 gal. of No. 1 tallow. Condemnations ran about 20% of the flocks with 80–90% condemnations at times. The affected chickens showed large accumulations of pericardial fluid with associated liver damage. Separate tests absolved crotolaria seed and pathogenic organisms as alternate possibilities.

The nature of this outbreak and other information to date makes it seem probable that fats entering fatty acid plants at times contain the C.E.F. in marginal quantities, which become concentrated by various processing steps.

Since the finding of a chlorinated pesticide in C.E.F. positive fat, further work showed Chlordane to be present in a specially concentrated edema toxic fatty acid prepared from some toxic residue material.

Recent industry evidence substantiates the earlier findings of the FDA that vegetable fatty acids also contained the C.E.F. at times. This eliminates the necessity of postulating the animal body as a metabolic intermediate in the production of the C.E.F. from some chlorinated precursor.

In an article to appear shortly in the Journal of Poultry Science, Dr. O'Dell, *et al.*, of the University of Missouri, will report on an occurrence of chick edema indistinguishable from that of edema positive fats, which he traced to a chlorinated biphenyl compound incorporated into an epoxy paint and mixed with sand (15). He had coated the bottom of his galvanized chick batteries with this composition, and the chicks pecking on the sand picked up the toxic factor. The chlorinated biphenyl material responsible for this edema toxicity was found to be Aroclor 1242. Dr. O'Dell reports his belief that the active ingredient is a minor constituent in the Aroclor 1242.

This product is substantially biphenyl, with 42 weight per cent of chlorine. Among uses for this material suggested by the manufacturer is one as a vapor suppressant for insecticides, with from 5 to 25 weight per cent of the chlorinated biphenyl increasing the effective kill life of Lindane by a factor of 10. I quote the following from the manufacturer's bulletin on Aroclors. "The most pronounced effect for increasing the kill-life of the insecticide is obtained with Lindane, Chlordane and BHC" (16).

Is this recommendation a coincidence, or is it related to our finding chlorinated pesticides associated with edema toxic fat? The chlorine content is of the order of magnitude of the purified C.E.F.

Our Association is currently trying to track down these leads.

In addition to these problems concerned with the nature of the C.E.F., the industry has been confronted with the onerous task of feeding chickens for three week intervals as a quality control check on finished food grade acids. You can imagine the scheduling and storage problems entailed.

We are trying to work out an acceptable diet and criterion for deciding what constitutes a positive finding of "edema" by collaborative work with the FDA. Originally, chick feeding tests were run using a laboratory procedure provided by the FDA. It was known as the "Friedman" test. This test used visual scoring to determine an edema positive chicken. A modification requiring the presence of a definite fluid level was an improvement.

Numerous workers were not satisfied with this test for various reasons, so a collaborative study was made using a diet having higher salt levels designed to increase the sensitivity. The diet also was nutritionally better. It was issued early in 1961 as a tentative A.O.A.C. procedure (17). This test again called for visual scoring, but in work with our association, the FDA picked a level of 0.2 ml of heart fluid on a single chick in 10 as a positive response.

Using this procedure a number of laboratories began finding what appeared to be false positives on control fats. This would be indicated by a single chick showing a level of heart fluid of over 0.2 ml while the balance of the test group would have very low levels. On rechecking, the positive finding could not be substantiated. This posed the question whether the "positive" chick was abnormally sensitive to the C.E.F. or to the high level of salt in the diet. An interesting discussion of sodium chloride levels and their relation to chick hydropericardium was given by Dr. Alexander at the Poultry Association meeting held in August of this year (18).

After another collaborative survey during 1961 using essentially the same diet, the FDA have proposed a statistical value for deciding what consitutes a positive response. This is now being reviewed by our industry.

The Fatty Acid Producers Council currently have before the FDA a petition for a regulation covering fatty acids and derivatives thereform. It is expected that the regulation will not issue until problems concerned with the chick bioassay procedure are cleared up. The petition includes a reservation on the requirement for running any type of test for the C.E.F.

It would seem from the evidence available to date that the presence of the C.E.F. in *food grade fatty acids* is an unusual and rare occurrence and our industry should not be required to test for this factor as a routine matter any more than we should be required to test for thousands of other toxicants which might accidentally get in. This should be a matter for careful production and is already a responsibility spelled out in section 402 of the act applying to adulterated foods. The problem should be looked at from a broader frame of reference than only the fatty acid industry.

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[Received November 1, 1961]

U.C.L.A. Announces Gas Chromatography Course

A Gas Chromatography Course will be held at the University of California, Los Angeles, January 31 to February 2, 1962. This intensive three day course is the fourth basic course presented in response to requests from industry. The first course was held in February 1959, the second in June 1959, and the third in August 1960. This fourth course has been redesigned and brought up to date with emphasis on recent developments and applications.

The course is basic rather than advanced. It is aimed primarily to instruct personnel from industry, although it may be equally valuable to persons in academic and government laboratories. Enrollment will be limited, with a bachelor's degree in science or engineering, or equivalent, required as prerequisite. The course will consist principally of lectures, augmented by adequate laboratory demonstrations. The fee for the course will be $100; additional information and application blanks may be obtained by writing to H. L. Tallman, Room 6532, Engineering Building, University of California, Los Angeles 24, Calif.