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 unsaponifiable material was unexpected. These oleic acids were found to abnormally affect rats and monkeys, and 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 compound 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. There was a feeling at the time that the distillation somehow was the appearance of the C.E.F. One group in the Fatty Acid Industry felt the effect was more 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 break with low grade inedible tallow that the FDA spelled out the edible requirement for monoglycerides although section 402 of the act covered this matter.

While no evidence had appeared that stearic acid had C.E.F., it was omitted from the GRAS classification because of the possibility that it could be produced from oleic by low pressure hydrogenation, which was known to affect the C.E.F. (4). In other words, the possibility placed on the manufacturers to define stearic acid that was safe for food use, rather than to define the chemical product generally under the GRAS concept.

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 now that the time interval allowed to get information about the 1960 enforcement deadline by petitioning for a regulation.

The fatty acid industry set in motion a very intensive search for chick edema positive materials. Testing and sterilizing hydrocarbon diene concentrates, cholesterol diene and chick feeding tests, were among the numerous work samples of C.E.F. positive [illegible] furnished by D.P.L. rapidly established the [illegible] diene, the cholesterol diene and hydrocarbon diene tests being chick feeding tests to fall back on, which [illegible] where we stand today.

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

In the [illegible] of 1960 while the fatty acid industry was searching its supplies and processes [illegible] for material, the Food and Drug inspectors [illegible] of both acids and derivatives in the various producers, for their own evaluation.

The upshot of all this activity was that [illegible] with negative results. No C.E.F. appeared [illegible] even in first, second, or third [illegible] oleic or cottonseed acids, much [illegible] the distillates, stearic acids. This threw cold water on the [illegible] tillation theories for the C.E.F. formation.

At about this time, the Association became [illegible] with the concentration and isolation work on the C... being done by a group of workers at Merck & Co. and Proctor & 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 biological assay 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 starting edema positive tallow. Help was asked of the association members in supplying more concentrated source 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. Brenner 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 non-aromatic molecule, having from 10–12 carbon atoms, 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.

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200°C. A mole[illegible] weight of approximately 380 was indicated. This ag[illegible] 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 w[illegible] were convinced that they [illegible]re dealing with an un[illegible] material resulting from contamination and decide[illegible] continue any further work. Before doing [illegible]o, th[illegible] number of chlorinated cyclodiene pesticides, as [illegible] ber of the members of our Association, [illegible] FD[illegible] ter & Gamble. Also checked were chlorinated n[illegible] which had been implicated in toxic effects on [illegible] years before. The workers at the FDA [illegible]eaned [illegible] chlorinated naphthalene theory for the C.E.F., [illegible] their data. The pesticide theory for the C.E.F. [illegible] most plausible interpretation of the Merck results. To check these possibilities, several pesticides and lubri[illegible]nts 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, Heptachlor, Hexachlorophene, tetrachlorotetrahydron[illegible]phthalene, hexachlorocyclohexane, pentachlorophenol, tetr[illegible]lorotoluene and hexachloronaphthalene.

The Association also sponsored small [illegible] ren[illegible] at the American Meat Institute using [illegible] hav[illegible] Dieldrin, Endrin and Heptachlor added [illegible] lev[illegible] to give 10 p.p.m. of contaminant in the [illegible]ered [illegible] were all found to be negative to the [illegible]edin[illegible] tests on the rendered fats showed 100% [illegible]orta[illegible] case, indicating the presence of the [illegible]se [illegible]ides tests by the same company on known C.E. pos[illegible] and oleic acids had shown no insecticidal activity.

Another company ran the gamut of available [illegible] in testing for thermal breakdown products, by h[illegible] at a 1% level in fatty acids to over 200°C for ex[illegible] periods of time. In no case did a positive edema test [illegible] 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 [illegible] had this limitation, which the commissioner later re[illegible] [illegible] his own initiative, after we pointed out the dis[illegible]

In September of 1960 another outbreak ident[illegible] number of independent laboratories as "Chick E[illegible] sease" occurred in some brooder houses in Geor[illegible] C.E.F. positive feed concentrate was traced to th[illegible] tion of a single day. Unfortunately, neither the fat [illegible]ner nor the feed manufacturer retained fat samples, [illegible] not been possible unequivocally to prove that cont[illegible] fat, which in this case was No. 1 tallow, was the [illegible] various other feed components were checked by fee[illegible] 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

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nontoxic feeds taken from production about the same time showed a few p.p.m. of Chlordane, [illegible] 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 cytolaria 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 toxin 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 as 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 [illegible] low levels [illegible] the positive finding [illegible] question whether [illegible] sitive to the C.E.F. [illegible] An interesting [illegible] relation to chick [illegible] under at the Poultry [illegible] of this year (18).

After another collaborative survey during 1961 [illegible] essentially the same diet, the FDA has proposed a statistical value for deciding [illegible] This is now being reviewed by our industry.

The Fatty Acid Producers Council [illegible] before the FDA a petition for a regulation covering [illegible] acids and derivatives thereform. It is expected that the [illegible] will not issue until problems concerned with the [illegible] assay 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* [illegible] unusual and rare occurrence and one [illegible] be required to test for this factor as a [illegible] more than we should be required to test [illegible] other toxicants which might accidentally get [illegible] be a matter for careful production and is already [illegible] sibility spelled out in section 402 of the act applying to adulterated foods. The problem should be looked at in a broader frame of reference than only the fatty acid industry.

REFERENCES

1. Effect of Certain Compositions and Source Variables on Wholesomeness of Fat for Feed—by Hathaway R. D., Burnett, C. M., and Patterson, R. L. Feedstuffs, January 10, 1959.
2. Symposium reported in Journal of the Assn. of Off. Agric. Chem., 130–148, February 1959.
3. Brew, W. E., Dore, J. B., Benedict, J. H., Petty, R. C., and Sipos, Endre. *Ibid.*
4. Friedman, L., Firestone, D., Horwitz, W., Benson, D. Arnold, M., and Rhee, G. *Ibid.*
5. Wooten, J. C., and Alexander, J. *Ibid.*
6. Potter, C., Brew, W. E., Patterson, R. L. and Sipos, E. J. Am. Oil Chemists' Soc., 38, 214–217 (1961).
7. Ames, S. R., Swanson, W. J., Ludwig, M. and Brekke, J. Am. Oil Chemists' Soc., 37, 10–11 (1960).
8. Ames, S. R. Federation Proc., 19, 425 (1960).
Am. Oil Chemists' Soc., 37, 10–11 (1960).
9. Firestone, D., et al., J. Am. Oil Chemists' Soc., 38, 418–422 (1961).
10. Tarkoff, A., Firestone, D., et al., J. Am. Oil Chemists' Soc., 36, 60–63 (1961).
11. Harmon, R. E., Davis, G. E., Ott, W. H. [illegible] O. Knehl Jr. F. A., *Ibid.*, 38, 3078–9 (1960).
12. Ott, W. H., et al., J. Poultry Science, 40 [illegible]
13. Kirk, J. K., J. Am. Oil Chemists' Soc., 37 [illegible]
14. J.A.O.A.C., 44, 147–7 (1961).
15. McCune, E. L., Savage, J. E., and O'Dell, Preparation report. Univ. of Mo.
16. Aroclor Compounds—Monsanto Chemical Company Bulletin 0860-S8 p. 17. See also Aroclor Plasticizers Bul. PL-606.
17. J.A.O.A.C., 44, 146–7 (1961).
18. Alexander, J. C., 50th Annual Meeting, Poultry Science Assn. August 1961.

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

A Gas Chromatography Course will be held at the University of California, Los Angeles, January 2, 1962. This intensive three day course is the [illegible] course presented in response to requests from [illegible] The first course was held in February 1959, the second 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 personnel 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.

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