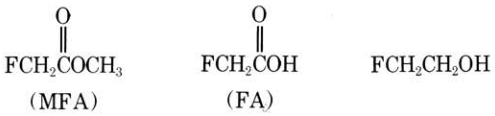
Gordon W. Gribble[1]
Dartmouth College
Hanover, New Hampshire 03755

Fluoroacetate Toxicity

Under the stress of World War II chemists in England, Germany and their allied countries sought to develop chemicals (independently, of course!) which would incapacitate, maim, or kill the enemy. These remarkably successful researches led to the synthesis and large-scale production of several types of warfare agents: nerve gases, vesicant agents, tear gases, harassing compounds, and, perhaps the most frightening of all, water poisons.

For the latter kind of chemical agent it can be easily envisaged that a secret agent could poison the water supply of a large enemy populace with but a small amount of a toxic chemical. The requirements for a water poison are stringent: it should be colorless, odorless, soluble, stable, and highly toxic, preferably with a delayed action to prevent early detection. It therefore must have come as quite a surprise to chemists in England, Germany, and Poland when they discovered independently during the early stages of the war that a simple derivative of acetic acid fulfills all of the above criteria for an ideal water poison!

This compound is methyl fluoroacetate (MFA) and it, along with fluoroacetic acid (FA) and 2-fluoroethanol, represents one of the most toxic classes of non-protein substances known.



As seen in Table 1 these compounds are more toxic, on a per weight basis, than several other well-known deadly poisons.

Preparation and Properties

MFA was first prepared in 1896 by the Belgian chemist Swarts (*1*) by treatment of methyl iodoacetate with silver fluoride, a rather expensive and inefficient procedure.

$$ICH_2CO_2CH_3 + AgF \longrightarrow FCH_2CO_2CH_3 + AgI$$

MFA, because of its great toxicity, came into consideration at the start of World War II as a potential warfare agent (*2–4*). MFA seemed especially suitable as a water poison because of its "ideal" physical and chemical properties for this type of agent (see Table 2). In addition, its toxic action is delayed making early detection of MFA in water supplies difficult.

By the end of the war several countries including England, the United States, Poland, and Germany had developed efficient pilot-plant methods for the preparation of MFA.

Saunders and his colleagues in England used a rotating autoclave at 220°C to produce MFA from methyl chloroacetate and potassium fluoride in 54% yield (*5*). A similar method was developed by a Polish group (*6*). Schrader in Germany found that ethyl diazoacetate and hydrofluoric acid furnished ethyl fluoroacetate (*7*). Several other syntheses of these fluoro esters were disclosed after the war (*2*).

Table 1. Toxicities of MFA and Other Toxic Compounds[a]

Compound	LD_{50}(mg/kg) rats[b]	LD_{50}(mg/kg) mice[b]
MFA	...	6
FCH_2CO_2Na	0.2	...
mustard gas	0.7	...
parathion	10	...
strychnine	5	0.5
NaCN	...	10
DFP (nerve agent)	...	4

[a] Taken from several sources.
[b] The dose required to kill 50% of the animals by subcutaneous injection.

Table 2. Selected Physical Properties of MFA[a]

Property	Value
boiling point	104° (760 mm)
melting point	−32°
water solubility	15%
odor	faintly fruity at 10 ppm

[a] Reference *(4)*.

Unlike the other haloacetates MFA is remarkably resistant to displacement of fluoride by nucleophiles. For example, MFA when refluxed for 1 hr with 10% NaOH gives no fluoride. After 20 hr of reflux with 20% KOH only 50% of fluoride is liberated (*5*). Similarly, ethyl bromoacetate is at least 400,000 times more reactive than MFA towards sulfite ion. This inherent stability of the C-F bond in MFA must also account for the absence of lachrymatory properties for MFA, unlike the chloro-, bromo-, and iodoacetates which are acute tear-producing agents. These haloacetates act as powerful alkylating agents, like mustard gas and dimethyl sulfate, and react with cellular nucleophiles such as the SH group of proteins and the nucleophilic nitrogens of nucleic acid bases. In stark contrast to these haloacetates, MFA is not a biological alkylating agent and, in fact, the C-F bond in MFA remains *intact* throughout the course of poisoning! Thus, paradoxically, it is the great stability of MFA and FA which leads to their unique toxic action. What then is the explanation for the great toxicity of MFA and FA?

Toxic Action

Several investigators in 1948–49 observed that citric acid accumulated in the tissues of MFA-treated animals (*8–10*). It was then logically suggested that FA entered the citric acid cycle (Krebs) and somehow prevented the further utilization of citric acid (*11, 12*).

Before exploring the exact nature of this interplay of FA with the citric acid cycle let us review the latter (*13*). The role served by the citric acid cycle is to oxidize fatty acids, carbohydrates, and amino acids. To do this, these foodstuff molecules must first be degraded to acetic acid (in the form of acetyl coenzyme A). Finally, acetic acid is oxidized according to the following equation.

$$CH_3CO_2H + 12P_i + 12ADP + 2O_2 \longrightarrow 2CO_2 + 14H_2O + 12ATP$$

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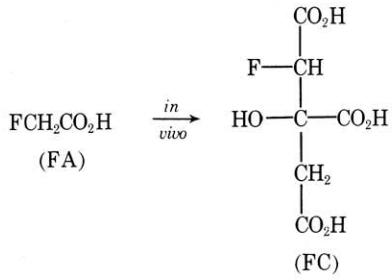
The importance of the citric acid cycle, shown in its entirety below, cannot be overstated since the energy of one acetic acid molecule is transformed and conserved in the form of 12 ATP molecules which in turn serve as essential energy carriers in living cells.

Returning to the mode of action of FA, it was observed that FA does not inhibit any isolated individual citric acid cycle enzyme (*11, 14*). This would appear to demand the conclusion that FA is converted *in vivo* to a different, more toxic substance which enters the citric acid cycle. Peters has provided convincing evidence that this toxin is fluorocitrate (FC) which is synthesized *in vivo* from FA (*11, 14*).



The FC subsequently inhibits the enzyme aconitase, which is concerned with the conversion of citric acid to aconitic acid and/or isocitric acid. This inhibition leads to a fatal buildup of citric acid in the tissues, culminating in violent convulsions and death from cardiac failure or respiratory arrest (see Fig. 1).

The evidence for Peters' proposal is quite strong: (1) FC can be isolated from FA poisoned animals; (2) FC inhibits highly purified aconitase whereas FA does not; and (3) acetate exhibits a protective effect, presumably by interfering with the conversion of FA into FC.

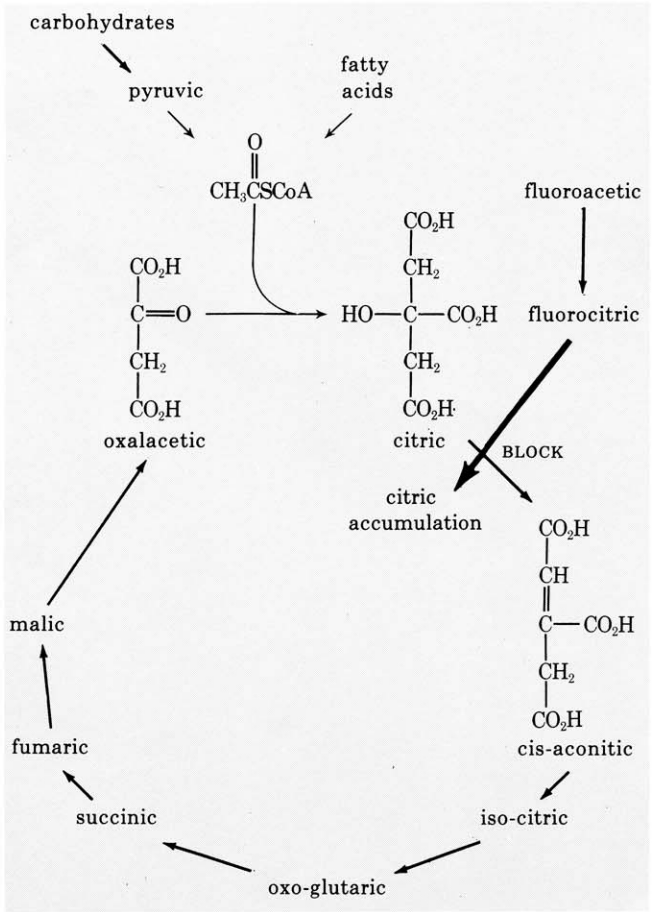


Figure 1. Citric acid cycle.

In addition, structure-toxicity studies provide further evidence in support of the FC hypothesis.

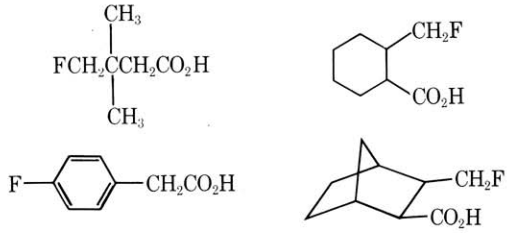
Structure-Activity Relationships

One might reasonably predict that a compound capable of *in vivo* conversion to FA would be toxic. Indeed, it is clear from Table 3 that this is exactly the case. Fluoroethanol and fluoroacetaldehyde can be oxidized *in vivo* to FA while fluoroacetyl fluoride, fluoroacetamide, and fluoroacetic anhydride can be hydrolyzed to FA. All are about as toxic as MFA. On the other hand, compounds such as difluoroacetic acid, chloroacetyl fluoride, and methyl fluoroformate are nontoxic since they cannot be hydrolyzed to FA. Not surprisingly, the chlorine atom in 1-chloro-2-fluoroethane is apparently not displaced by water in the organism and, as a result, is nontoxic.

Since only those fatty acids containing an even number of carbon atoms are degraded to acetic acid *in vivo,* it is found that only the corresponding ω-fluorocarboxylic acids are toxic. The odd-carbon ω-fluorocarboxylic acids are non-toxic. As seen in Table 4 the results are striking indeed.

The observed alternation in toxicity is a beautiful verification of the theory of β-oxidation of fatty acids, summarized in Figure 2 (*15*).

In accord with the β-oxidation theory as applied above to ω-fluorocarboxylic acids, the following derivatives are all non-toxic, even though they possess an even-numbered carbon chain. It is readily seen that in each case one or more of the steps in the β-oxidation theory is impossible.



In spite of the general understanding of FA toxicity, the details of the FC-aconitase inhibition are unknown although schemes have been advanced (*16*).

Natural Occurrence

At least three natural sources of FA and derivatives

Table 3. Toxicity of Selected Fluoroacetyl and Related Compounds[a]

Toxic[b]	Nontoxic[c]
FCH_2CO_2H	F_2CHCO_2H
FCH_2CO_2R	F_3CCO_2H
FCH_2COF	$ClCH_2COF$
FCH_2CHO	FCH_2CH_2Cl
FCH_2CONH_2	CH_3CHFCO_2H
$(FCH_2CO)_2O$	$(CH_3)_2CFCO_2H$
FCH_2CH_2OH	FCO_2CH_3

[a] References (*2–4*)
[b] Toxicity comparable with that of MFA.
[c] No MFA-like toxicity.

Table 4. Toxicity of ω-Fluorocarboxylates[a]

Compound	LD_{50}(mg/kg) mice	Conclusion
$FCH_2CO_2CH_3$	15	toxic
$F(CH_2)_2CO_2C_2H_5$	>200	nontoxic
$F(CH_2)_3CO_2CH_3$	. . .	toxic
$F(CH_2)_4CO_2C_2H_5$	>160	nontoxic
$F(CH_2)_5CO_2C_2H_5$	4	toxic
$F(CH_2)_{10}CO_2C_2H_5$	>100	nontoxic
$F(CH_2)_{11}CO_2C_2H_5$	20	toxic

[a] References (*2–4*).

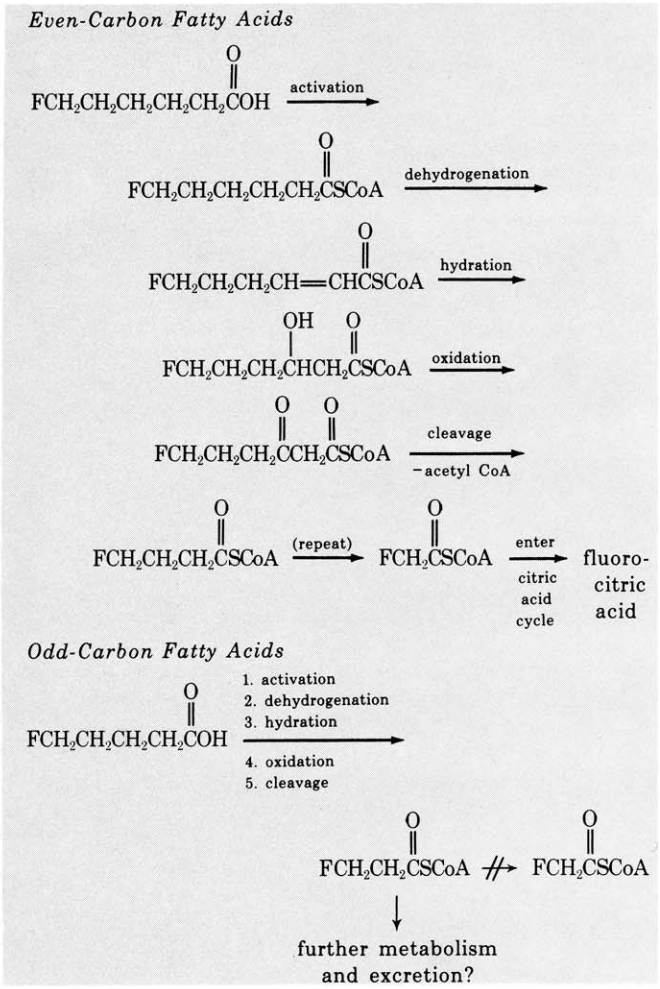


Figure 2. β-Oxidation of fatty acids.

have been discovered to date. The South African plant gifblaar (*dichapetalum cymosum*) contains potassium fluoroacetate (*17*), the Sierra Leone shrub ratsbane (*Dichapetalum toxicarium*) seems to contain a ω-fluorooctadecenoic acid (*18*), and the Australian plant *Gastrolobium grandiflorum* has present in its leaves fluoroacetic acid (*19*). Less than an ounce of gifblaar leaves is enough to kill a sheep, and it is claimed that one half a leaf is fatal to an ox (*4*).

Another interesting aspect of FA toxicity is its remarkable species specificity. For example, MFA is highly toxic to the Texas pocket gopher ($LD_{100} < 0.05$ mg/kg) but not to the South African clawed toad ($LD_{50} > 500$ mg/kg) (*20*).

Treatment

As mentioned earlier, large doses of acetate or an acetate source such as glycerol monoacetate or ethanol help to prevent the lethal synthesis of FC from FA. However, this is, in effect, preventive treatment and is effective only if administered immediately after poisoning and thus prior to the FC synthesis and citrate accumulation. In view of the fact that citrate is reasonably effective in lowering the Pb(II) concentration in lead poisoned animals (*21*), it would be interesting to see if Pb(II) treatment can lower the citrate buildup and prevent the fatal onslaught of convulsions which result from high citrate concentrations in the tissues. Citrate forms a strong complex with Pb(II) which apparently is readily excreted. Since citric acid accumulation probably disturbs the normal calcium ion balance in the organism another treatment which is therefore suggested is administration of calcium ion. This may help to restore the normal calcium balance by removing excess citrate through complexation.

Since sodium fluoroacetate is used commercially as a rodenticide (trade name: "1080") proper caution should be exercised in its handling as several cases of human poisoning have been recorded (*22–24*). The lethal dose for a 150 lb man is estimated to be about 400 mg (*25*). Particular attention should be paid to the fact that the fluoroacetate and fluorocitrate residing in the carcass remains toxic for a long period, unlike most other organic poisons.

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