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PRELIMINARY REPORT ON
A STUDY OF THE FACTORS RESPONSIBLE
FOR THE TOXICITY OF WASTES FROM
A MODERN KRAFT PULP AND PAPER MILL

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SUMMARY

The wastes investigated were obtained from the main sewer of a modern Kraft paper mill, pulping only Douglas fir. After chemical and physical treatment in the laboratory that completely removes inorganic sulfide, hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, the toxicity of the waste remains essentially undiminished. Other chemical and physical treatment of the waste, designed to precipitate or otherwise inactivate soaps indicates that neither fatty-acid soaps nor the so-called "resinacid" soaps are much concerned in producing the toxic effect on fish.

The toxic agent is not volatile at 100° C. (at which temperature Kraft waste does not boil). When the waste is distilled at approximately 101.5° C., its average boiling point, the toxic factor (or factors) is completely removed in the steam. The distillate has roughly the same toxicity as the untreated Kraft waste, and produces the same symptoms in the fish. The colorless distillate contains a clear, oily liquid which floats on the surface in water-clear droplets, and is suspended throughout the distillate in droplets of approximately colloidal size.

The poisonous material (or materials) is completely removed from the distillate, or from whole Kraft waste, by extraction with ethyl ether. Investigation of its nature is continuing. It certainly contains one or more terpenes, probably fatty acids, perhaps other compounds. Some observations of its physiological effect on salmon have been made.

INTRODUCTION

The wastes investigated were obtained from the main sewer of a modern Kraft paper mill, pulping only Douglas fir. Most of the black liquor solids, and essentially all of the solid "resin acid soaps" are destroyed by burning, and the volatile condenser and reliever wastes are passed through a Bergstrom tower, which removes much of the volatile sulfides content (mercaptans, dimethyl sulfide, hydrogen sulfide). The main sewer contains all of the waste water from the Kraft operation, with the exception of a small amount of runoff from the floors of the various buildings, which is run separately to settling ponds to minimize danger from spills.

Even though the waste reaching the main sewer has been treated so that the content of inorganic sulfide, mercaptan, soap and black liquor is greatly reduced, it is still quite toxic to silver salmon (*O. kisutch*). Salmon are killed at dilutions of 1 in 10 nearly always, at dilutions of 1 in 20 most of the time, and at 1 in 40 perhaps half of the time.

KRAFT SYMPTOMS

Fish poisoned by these Kraft wastes invariably develop a characteristic set of symptoms; no fish has yet been observed (out of hundreds tested) which died, or was severely affected by Kraft liquor, without showing them. So constant and unvarying are these symptoms that we refer to them simply as "Kraft symptoms".

The striking feature of "Kraft symptoms" is the apparent inability of the fish to close the gill-covers or opercula. In strong solutions of waste, the opercula cease to close completely within a minute or so; in dilute ones the symptoms may take several hours to appear. Once established, the symptoms persist until death, or until the fish is removed to pure water. The respiratory rate may increase, or it may not. Even in strong solutions, there may be no evidence of labored respiration until shortly before death. The mouth gapes widely, and like the opercula, does not close completely in severe poisoning. In some smaller fish, the gill covers stand out stiffly and become almost motionless. The fish become somewhat dull and insensitive to stimuli, but all reflexes are present and adequate, swimming and avoiding movements are normal though slower than usual, and there is no evidence of loss of equilibrium or other nervous failure until shortly before death, when the fish goes over first on its side, then on its back. Just before death, the gills are again closed completely in convulsive breathing movements, and the body usually darkens because of melanophore expansion. After death, the fish usually, but not always, assumes the typical "asphyxiation position", with mouth and gill-covers widely gaping.

Death occurs after a lapse of time corresponding roughly to the dilution of the waste; perhaps 1.5 hours at 1:1, 3 hours at 1:3, 24 hours at 1:9, 48 hours at 1:19, and 3-5 days at 1:39. The toxicity of waste within the same sewer varies from sample to sample and from month to month; hence experiments have been confined to single lots of waste: each experiment has whole waste controls taken from that sample only; treated wastes can be compared only with their own controls, and not with controls (or other treated samples) taken at different dates from the same sewer.

SULFUR COMPOUNDS

Published American data, based mostly on mills pulping Eastern pines, had tended to regard sulfides, mercaptans and "resin acid" soaps as the most likely lethal compounds, and bioassays had established some minimum lethal doses of sulfide and mercaptan for silver salmon (Haydu et al., 1952).

Dilution alone seemed to eliminate sulfide and mercaptan. Both are usually present in amounts of less than 10 p.p.m. The minimum lethal dose for both is 1-5 p.p.m. Diluted 20-40 times, waste containing less than 10 p.p.m. of both regularly killed fish in samples of "average" (year-around average) toxicity.

To eliminate these volatile compounds, whole Kraft waste was heated to about 100° C. for 1 hour, and a vigorous stream of nitrogen gas was blown through the waste for the entire time. Tests made on water and Kraft waste blanks to which were added pure methyl mercaptan and dimethyl sulfide at 100 ± p.p.m. concentration showed that this treatment completely removed both compounds; and, when acidified to pH 1-2 with sulfuric acid, it removed inorganic sulfides as well. In the latter case, the pH was re-adjusted with NaOH. The addition of Na and SO₄ ions is not important, as sodium sulfate has been shown to be harmless to fish at 12,000- p.p.m.

Complete removal of inorganic sulfides, methyl mercaptan and any dimethyl sulfide that might have been present had no perceptible effect on the toxicity of Kraft waste, run against Kraft controls from the same sample. Another (superfluous) control from the same Kraft sample was heated to 100° C., but not treated with nitrogen. This was just as toxic as the other two in all cases.

These results indicate that the poisonous ingredient of this particular Kraft mill waste is not inorganic sulfide, methyl mercaptan, hydrogen sulfide or dimethyl sulfide.

DISTILLATION

Distillation of whole Kraft waste was resorted to as a means of separating the waste into fractions. Two liter samples were distilled from a 7-liter Erlenmeyer flask. The neck of the flask was tightly plugged with glass wool, to stop gross splashing and bubbling. Above the glass wool was a 1-inch air space below the stopper. A glass tube through the rubber stopper led to a vertical glass pipe, 2 inches in inner diameter and 18 inches high, stoppered above and below and packed with Raschig rings to a depth of 14 inches, there being a 4-inch space above the rings. A glass tube from the top of the glass pipe led to a water-cooled glass condenser tube, which led to the collecting vessel.

The Kraft waste boils at 101.5° C. More than nine-tenths of the waste was distilled over at each distillation. The distillate is water-clear, with a faint opalescence (and Tyndall effect) showing the presence of finely divided particulate matter throughout. At the surface, a water-clear, oily liquid collects in flattened droplets. This distillate is always about as toxic

as the original Kraft waste; that is, the toxic substance or substances belong to the class of compounds distillable with steam. The residue is dark brown in color, with much solid matter precipitating out. When made up to the volume (of waste before distillation), it is nearly harmless, and produces no Kraft symptoms at dilutions of 1:9.

What compounds might be present in Kraft waste that are distillable with steam? Assuming that the severe Kraft process has not broken down any of the following substances, careful analyses of Douglas fir wood (Guenther, 19) indicate that these steam-distillable compounds might be present:

- alpha pinene
- ~~beta pinene~~
- alpha terpineol
- fatty acids of less than 10 carbon atoms

The following compounds not distillable with steam might be expected in the residue:

- oleic acid
- linoleic acid
- linolenic acid
- "rosin acids" so-called, related to abietic acid
- alkaline lignin
- inorganic salts

Actually, at least oleic acid breaks down on occasion to form two 9-carbon acids, which would be distillable with steam. Also, it should be remembered that no still is 100% effective--perhaps 1/1,000 of the weight of any of the above compounds is likely to come over. The very low toxicity of the residue, however, argues against very much of any of them being present in the residue, except, of course, the alkaline lignin and inorganic salts.

ETHER EXTRACTION

Two extractions with ethyl ether (200 cc. ether to 1 liter of waste) remove the toxic element completely from the distillate and from whole Kraft waste as well. If the extracted distillate and waste are treated with heat and nitrogen as in the removal of volatile sulfides, all of the ether can be driven off, and the distillate and waste are then perfectly harmless to salmon. On evaporation of the ether, a residue is obtained which is partly oily, partly waxy in consistency, varies in color from white to light brownish, and has a faint but definite odor of "pine" plus a rather dull odor difficult to describe, faintly like that of baked beans. This residue is slightly if at all soluble in

water, but can be restored to solution by use of some of the residue from the distillation (alkaline lignin plus some soap) and gentle heat. Presumably it is emulsified by the alkaline lignin (which is a detergent) in the Kraft waste. Heating is well known to break such emulsions. The re-emulsified residue is toxic to fishes, and produces Kraft symptoms. That it is not as toxic as the original waste is due doubtless to oxidation, loss in handling, and so on. It is, however, the only really poisonous fraction. Vigorous shaking and stirring with a glass rod will disperse some of it in a muddy-looking suspension of coarse particles which also kills fish with Kraft symptoms without any emulsifying agent.

THE SOAP QUESTION

Both fatty-acid and "resin acid" soaps are probably present in Kraft waste. Two methods of testing their contribution to toxicity were used: precipitation as insoluble calcium soaps and distillation of waste from strongly acid and alkaline media.

Precipitation

Two grams of CaO were added to each liter of Kraft waste, stirred, and allowed to settle for several hours. Not only does this treatment presumably convert the soluble Na-soaps to insoluble, precipitated Ca-soaps, it also precipitates all of the visible organic matter (lignin, etc.), leaving a clear supernatant. This clear fluid was removed by siphon, and gaseous CO₂ blown through it to precipitate the excess CaO and to bring the pH back into the normal Kraft waste range (c. pH 9). After the precipitated carbonate settled, the clear supernatant was again removed. Kraft waste so treated loses much of its toxicity, often not killing fish at 1:4 dilution. The fish are, however, severely affected by Kraft symptoms at dilutions down to 1:9.

If the precipitated residue thrown down by the CaO is washed with water, made up to original volume, and distilled, the distillate is quite toxic, and produces Kraft symptoms. It would thus seem that:

1. CaO does not precipitate the toxic factor as such, but does precipitate other compounds (lignin, soap) which may have formed emulsifying micelles with the factor, which is thus carried down mechanically.
2. The toxic agent thus carried down as part of an emulsion is released from the emulsion, at least partly, by heating and reappears in the distillate.

3. Not all of the toxic factor is emulsified. Some is probably in true solution and not carried down in the emulsion. Monoterpenes are slightly water-soluble, and the solubility of lower fatty acids varies from good to poor. "Resin" (abietic acid, etc.) acids are insoluble in water.

Distillation

It will be recalled that in distillation of whole Kraft waste the residue, presumably containing the soap, had little toxicity. Since it is generally accepted that soaps of more than 10 carbon atoms (e.g., oleic) are slightly, if at all, distillable with steam and also that the toxic action of soap is due mostly to free fatty (or other) acid formed on hydrolysis, it was thought that light might be thrown upon the role of soaps in Kraft toxicity by distilling Kraft waste made up to very acid (1-normal with H_2SO_4) and very alkaline (1-normal, with NaOH) reactions, and comparing the toxicities of wastes so distilled with that of a distilled untreated Kraft waste sample (pH about 9).

In the acid solution, the soap should break down almost completely to free fatty or "resin acids", which presumably would distil over more readily with steam, increasing the toxicity of the distillate.

In the alkaline solution, soap should be held as soap, no free acid should be present, and the toxicity of the distillate should be less, if soap is concerned at all.

The result of these treatments of whole Kraft waste was that no detectable difference appeared in the distillates of acid, alkaline or untreated waste. On several grounds, then, soaps do not seem to be important factors in the toxicity of the waste from this mill. This is probably because there is not much soap in the waste, since the pure soap itself is highly toxic to salmon, as will be discussed below.

EXPERIMENTS WITH CONCENTRATED SOAP

A sample of crude sulfate soap was salvaged from the duct leading to the burner. It was very impure, containing much wood fiber, lignin and considerable inorganic sulfide. This soap could be dried in an oven at $60^{\circ} C$. for a week, to form a leathery black mass which killed salmon with Kraft symptoms at concentrations of a few parts per million. No exact weights were kept, because of the impurity of the sample.

It was decided to inject some of this soap into the bodies of young salmon, to see what symptoms would be called forth. A heavy suspension of the dried crude soap was made in water, and injected into the body-cavity of a 4-inch silver salmon. There is room in the body cavity of such a fish for only about 0.4 cc., and this amount was used as a standard for fish of this length, arbitrarily prepared suspensions of soap being used. The fish did not die. The body wall covering the body cavity turned black, presumably because the soap seeped through the wall to expand the melanophores on the outer surface. The fish sank to the bottom, was obviously distressed, breathed with difficulty and lost its equilibrium somewhat. In a few hours it had recovered completely. Nine other fish were treated in the same way. Two died, the others recovered. When the same 0.4 cc. of soap suspension was squirted into 5 liters of water (diluted more than 10,000 times), salmon of the same size placed in jars containing this solution were killed in 6-8 hours, with typical Kraft symptoms. None of the injected fish showed Kraft symptoms, even the two that died; all of the fishes (12) tested with the dilute soap solution externally did.

This indicates (1) that crude soap can kill fish with Kraft symptoms, and (2) that the toxin acts only externally, not internally, probably affecting the gills. Probably the symptoms produced in the injected fish were due largely to the high sulfide content of the impure soap.

When a gram of dried soap was dissolved in a liter of water and distilled, a water-clear distillate resembling that produced by distilling Kraft waste was obtained. This also had a layer of clear oily droplets at the surface of the water. The odor, however, was much different, the distillate having a strong smell characteristic of this sample of crude soap. Ethyl ether extraction of the dry soap yielded a light brown substance similar to that obtained from whole Kraft in its toxic properties, but having the much stronger odor of crude sulfate soap.

This indicates that crude sulfate soap contains an ether soluble, steam-distillable toxic factor perhaps identical with the one in whole Kraft waste and Kraft waste distillate. The soap has some toxicity per se, and perhaps other poisonous substances with these physical properties occur in the soap as well. Only small amounts of the substance can be extracted from the soap with petroleum ether, most of it after repeated extractions with ethyl ether, and all of it with a single extraction with absolute ethyl alcohol. Since the alcohol dissolves soap as well, and in view of the fact that the ether extracted substance is insoluble in water in the absence of detergents (soap or

lignin), it is apparent that the compound (or compounds) is held in emulsion in the soap phase, which renders it soluble in water (as an emulsion) and which protects it from extraction with petroleum ether.

RESIN ACIDS

The Liebermann color reaction, or some modification of it, is often used quantitatively to estimate resin acids in Kraft waste. It has long been known to be inaccurate. Recently, the writer saw the unpublished results of an investigation carried out by a pulp mill chemist, for the purpose of estimating resin acids more accurately. Using his own method, a sample of turpentine showed 250 p.p.m. resin acid, the Liebermann color reaction 5,000 p.p.m.! A black liquor soap sample assayed 320,000 p.p.m. by his method, by the Liebermann method 500,000 p.p.m. The Liebermann reaction gives the same color reaction with many terpenes, including pinene and camphene (often major constituents of various wood turpentines). It seems likely that much of what has formerly been estimated as "resin acid" is actually terpenes of one kind or another.

Until recently, "resin acids" were mostly referred to abietic acid. Recent work (Harris, et al. 1948 a.b.c.d.e.f., Fowler 1954) indicates that abietic acid represents only about 15-20% of average "resin acid". At least 8 closely related compounds make up "resin acid", and several of these isomerize easily (e.g., on heating). Others form compounds with sulfur and other substances likely to be present in Kraft digesters.

It is evident that the idea of a single "resin acid", or "resin acid soap" is too simple a concept. At least half of the soap fraction consists of fatty-acid soaps, which are just as toxic, or perhaps more so, than are the "resin acid" soaps.

DISCUSSION

A number of compounds of diverse chemical structure (soaps, fatty acids, synthetic detergents) have been shown to be toxic to bacteria, other plants, and animals by absorption on surface membranes. The properties of the membrane are altered, often in a manner fatal to the organism, as a result. Purified southern pine turpentine, or "spirits of turpentine"--mostly pinene--has a similar affinity for mucus membranes of animals. It is used medicinally as an irritant, and the vapor from turpentine stills has caused human deaths by its effect on respiratory membranes.

In experiments on silver salmon, "Kraft symptoms" were produced by fatty acid (oleic), synthetic detergents (Tide, etc.), soap (in high concentrations), by crude sulfate turpentine and by spirits of

turpentine. Brief experiments with crude Kraft sulfate turpentine have demonstrated the presence of a large amount of some acidic substance which is saponified with dilute NaOH to form a substance not soluble in ether. After treatment with acid, this substance is completely ether soluble; it kills fish with Kraft symptoms. The turpentine thus contains a substance resembling an organic acid (it could be an ester).

Pure abeitic acid is not soluble in water, and does not cause Kraft symptoms. Fish kept in a strong suspension of it die in a day or so (perhaps because some is ingested), with indications of central nervous system involvement.

Fishes severely poisoned with Kraft waste usually recover in a few hours after removal to pure water. Neither histological examination of tissues, including the gills, nor examination of gills under the wide-field microscope has ever shown any indication of tissue damage, blood pathology, mucus coagulation or any other visible effects. Absorbed films of fatty acid, etc., would be so thin as not to be demonstrable by ordinary methods.

Previous studies of the toxicity of Kraft mill waste water in the United States have stressed sulfides, mercaptans and the so-called "resin acid" soaps. European workers had stressed "resin acids" and other "resinous" materials. Differences in the species of tree pulped, plant procedure, and the treatment of waste to remove soaps and volatile compounds, or the lack of such treatment, are so great as to preclude direct comparisons of plants operating in different countries, or in different parts of this country. The use of Liebermann-type color reactions for the determination of "resin acids" perhaps means that terpenes have often been identified as "resin acids".

The work of Danneel (1935) and of Ebeling (1930 a, b; 1932) on the physiological effects of terpenes on fishes have demonstrated a pattern of symptoms identical with those produced by the toxic agent in Kraft waste. They are harmless when injected into the body, kill in small concentration when applied externally, leave no visible or permanent evidences of harmful action on tissues or blood, and produce no central nervous system upsets. Fish recover rapidly when removed to fresh water. Symptoms resembling "Kraft symptoms" are described as affecting the respiratory movements.

Present indications are that the toxic fraction will be found to consist of terpenes, fatty acids and perhaps other compounds. Dimethyl disulfide is also being investigated, although, because of its very characteristic (stench) odor, it is not thought that it is present in the isolated toxic fraction, which has little odor, and that rather pleasant.

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