Andrew Balfour

21 Heriot Row

Edinburgh

THE TOXICITY OF THE DYE STUFFS.

With special reference to their effects upon the Public Health, and to river pollution.

By

Andrew Balfour, M.B.C.M. (Edin.) D.P.H. (Camb.)

I N D E X.

Historical Introduction on Toxicity of dyes in general.

The consideration of what are known as "the diseases of occupation" is undoubtedly one of much importance, from a medical, legal and commercial point of view. This being the case it is surprising how little attention has been devoted to the subject. Here and there we find a text book treating of it, and articles now and again appear, but the medical world has not greatly concerned itself with the question, though the columns of our newspapers frequently testify to its general interest. It is true that the ailments caused by certain trades have been carefully studied, that laws have been framed and regulations issued to prevent danger to individual health and to communities at large, but it cannot be said that careful attention has been paid to the whole subject of injurious occupations. Especially is this true of those industries which of recent years have rapidly grown to great dimensions, and which have altered in their methods and arrangements owing to the steady advance of science. In some instances the evil is not confined to the mere workers and those brought immediately into contact with deleterious substances, but affects the public health as well, and this is very clearly shewn in the

case of the great industry or rather industries having to do with the manufacture and use of dye stuffs. It is at once apparent that danger or annoyance may occur in several ways in connection with such a trade. Most people are aware that many dyes are virulent poisons, they have been taught that green is a dangerous colour, and they associate it with the presence of arsenic. They know that the salts of this metal are poisonous and cannot be handled with impunity, but few would readily suppose that contamination of the ground water by arsenic may occur and constitute a danger.

Again not only may the workpeople immediately concerned suffer in health from the action of dyes, but grave results may ensue from the inclusion of colouring matters in articles of dress, common commercial substances, or from their use in food stuffs and especially in confections. But this is not all, for we must bear in mind the frequent fouling of streams and killing of fish by effluents, indeed the amenity of many places has been ruined by the near proximity of dye works.

No doubt some attention has been directed to the subject and especially on the Continent a considerable amount of literature has accumulated, but it is found to deal chiefly with the older dyes, the

mineral salts, the early coal tar colours, and the vegetable juices, which have been ousted from the market by recent discoveries.

It is no easy matter to make researches on these new substances. Their number increases rapidly, they are complicated bodies, they are often impure, but many of them are in general use, and it seems but fitting that some knowledge of their toxicity should be forthcoming.

In reviewing the history of the subject it is well to consider the dye stuffs under the groups into which they have been divided, and they are at once found to fall under two great headings, the Inorganic and the Organic.

The latter again are divisible into Natural and Artificial, while all the groups may be further classed as to colour or chemical constitution.

I. Inorganic.

These are the dye stuffs whose toxic properties are best known, as they are derived from the common minerals, and have been before the public for a

long period of time. Some are comparatively unimportant, while others hold a foremost position in all works dealing with diseases of occupation.

Antimony in the form of the Sulphide is used to some extent as a dye, especially in indiarubber colouring, while tartar emetic is very largely used as a mordant in textile manufactories. All antimony salts have been found to be toxic, producing the well-known symptoms of vomiting and diarrhoea.

Arsenic is probably the most important poison found in the dye stuffs of this group. The green arsenical compounds are those usually to blame, and a great deal of attention has been directed to the subject. Poisoning, with the characteristic symptoms of vomiting, diarrhoea, and irritation of the urinary tract, affections of the skin and hair, and of the respiratory passages, has been recorded as arising from colours, dress fabrics, painted walls, wall papers, artificial flowers, the yarn in spinning works, and from many other sources, the fine arsenical dust contained in the dye being the exciting cause. Fatal cases are known to have occurred, chronic illness is far from uncommon, and at the present day instances now and again occur.

Brief reference may here be made to two interest-

ing and classical cases, the one, that of poisoning in a lady from whose green tarlatan ball dress Professor Erdmann obtained no less than 100 grains of pure arsenic, the other, several cases of arsenical poisoning in government clerks at Washington, U. S. A., resulting from the handling of "green back" dollar notes. Stockings, toys, and even cigarette holders, which have been coloured with arsenical dyes have been known to produce toxic effects, and instances might be given ad infinitum. In addition Arsenic from dye-works has been known to find its way into water supplies and streams, and to contaminate the ground water, while it exists as an impurity in many other dye stuffs.

Baryta. The Baryta colours, of which the best example is "Permanent White" ($BaSO_4$) are non-poisonous.

Cadmium. All Cadmium colours are toxic, the most commonly used being Brilliant Yellow (Cd S). Poisoning from this dye is however not common.

Chalk Colours such as Calcium carbonate are harmless.

Chromium. All the Chromium colouring matters are poisonous, evil results arising from the fine chrome dust which, as is well known, tends to attack mucous membranes, that of the nose most commonly suf-

fering. General irritant symptoms may occur. It is frequently associated with lead, and Weyl has recorded a case of yellow saddler's thread found to contain more than 21% of oxide of lead, existing as a chromate. Lehmann found chromium in yellow coach varnish, in the yellow varnish for milk pails, and even in penholders. Delpech and Hillairaret discovered lead chromate in an artificially coloured butter, and a case of pastry coloured in the same way, instead of by yolk of egg, has been recorded by Galippe (senior). Chrome green has been used to dye bank notes, and Bouchardet mentions that he found chromate of lead in a linen cloth covering American hams.

Copper. The salts of copper are well-known colouring matters, and bear an important relation to the public health from their use in the colouring of tinned vegetables. This rarely occurs in Britain, but is far from uncommon in France, where the process is known as Reverdisage. Peas are most commonly contaminated, and a case is recorded of from 180 to 270 m.g. of copper being found in 1 kilogram of preserved peas. The copper is said to exist in the form of copper-phylo-cyanate, copper leguminate, and to a less extent as copper oleate. Lehmann and Tschirsch believe one gram copper to be slightly toxic, producing

diarrhoea and vomiting in the human subject. On the whole, however, the copper colouring matters are comparatively innocuous. (Galippe, Toussaint, Moulin and Gautier).

Iron occurs chiefly in the Ochre dyes and is harmless except when contaminated with Arsenic, which is occasionally the case.

Lead. Of perhaps equal importance with the Arsenical dyes, are the lead colouring matters. The effect of the chrome salts of lead has already been considered, and all the coloured salts of lead are poisonous in small doses. The symptoms of plumbism are too well known to need description here, suffice to say that many epidemics have been recorded amongst lace workers and cotton weavers, and amongst the women employées in textile factories, where lead salts are not only used as dyes but as so-called "weighting" materials, and to hide defects in the fabric.

Manganese. Umbra is the best example of a Manganese colour. It consists of a mixture of the oxides of Manganese, Iron, and Aluminium, and is not poisonous. The same is true of the other Manganese dyes.

Mercury. All the mercury colours are poisonous or suspicious. Cinnabar was thought to be harmless,

but recent unpublished researches of Drs Marshall and Taylor of Cambridge have shewn that, if its action be continued for a long period, it is toxic. Like Arsenic it may be found in artificial flowers, &c., and it can be absorbed through the unbroken skin.

Tin. The salts of this metal chiefly occur in dyeing processes as mordants. They are slightly poisonous. The Sulphide $Sn S_2$ is that most commonly used.

Uranium. The salts of this rare metal are used in colouring processes to some extent. Their action has been investigated by Woroschilski and Chittenden, who have found them to be all poisonous.

Zinc. Zinc salts are chiefly used as mordants. They produce toxic effects something like those caused by Mercury and Lead, but they are not so dangerous and cannot be absorbed by the unbroken skin. Affection takes place through the alimentary and respiratory systems.

II. Organic.

a. Natural.

These consist of the dye stuffs derived from the vegetable and animal kingdoms, the latter group being chiefly obtained from the Insectae.

A considerable amount of work has been done in

connection with this division of the colouring matters, and it has been found that the great majority are non-toxic, so that their use is permitted in the preparation of food stuffs and confections, and in the painting of toys and fancy articles. At the same time their employment in the dyeing of textile fabrics has declined, as they have had to give way before the introduction of the more powerful coal tar colours. Those in most common use are here briefly mentioned, but further reference will be made in the experimental part of this paper, as regards the fouling of streams and their effects on fish. They are, perhaps, best classed under their colours, thus:-

White. Farina flour, starch, tragacanth, and white bone ash. All harmless.

Red. Cochineal, with or without cream of tartar, cherry and beet juices, various red fruit juices, infusion of red poppies. These are all harmless and are used largely by candy manufacturers, and in the painting of toys. Carthamic Acid derived from Saffron is said to be harmless, though Saffron itself has slight poisonous properties, which, however, seem to be due to its ethereal oil.

Blue. Colours derived from the expressed juice of blue flowers, such as litmus, bluebottles, violets,

&c. These are all non-toxic. A toxic colour has, however, been derived from Aconite, and its use is forbidden.

Yellow. Saffron when free from ethereal oil, safflower, turmeric, quercitron, extract from yellow woods, &c., are all harmless. Gamboge, however, has been known to cause toxic symptoms, and according to Wynter Blyth,has been used in the colouring of sweetmeats.

Orange. Annatto is harmless, and is commonly found in butter. Gamboge, mentioned above, may give an orange colour.

Green. Spinach juice and Chinese green are the most common and are both non-toxic. Many harmless greens are made from a mixture of yellow and blue dyes.

Violet. Archil and India wood, and fruit juices. All harmless. Solutions of natural indigo, which also supply blue colours, are harmless.

Brown. Caramel, Liquorice, extract of chestnut wood, and of catechu are non-toxic and in common use.

There is danger of some of these dyes being contaminated. This is probably greatest in the case of the "greens" from admixture of Scheele's, Vienna, or Schweinfurth greens, which contain Arsenic and are

very poisonous. They give a more vivid colour than the organic "greens."

Apart from this, Gamboge is practically the only dye which is to be feared and its poisonous properties have now been sufficiently recognised. They are of course of an irritant nature, drastic purgation being the most common symptom.

b. Artificial.

These at the present time constitute the most important of the colouring matters, and are practically all derived from coal-tar. Discovered about the middle of the present century, their use has advanced by leaps and bounds & every year sees numerous fresh dyes in the market. Their manufacture is now a great industry, especially in Germany, and they are much prized for the dyeing of cotton, silk, wool, leather, and various textile fabrics, on account of the vivid colours produced, their "fastness," durability, and cheapness.

The consideration of their toxic properties however, has by no means kept pace with their production and use, and it is only of late years that serious attention has been given to the subject. As already indicated, the work has been chiefly done on the Con-

tinent by German, French and Italian observers. Their researches have elicited many points of interest and importance, and corrected some erroneous ideas. Still a vast amount remains to be done, and the work is not easy for two reasons chiefly:-

a. Many of the dyes are wholly or comparatively insoluble in water and thus it is difficult to experiment with them.

b. It is not always easy to obtain them pure, and the existing impurities may be, and often are, poisonous substances.

In commencing this research it was at once apparent that what was chiefly required was an indication of the exact relative toxicity of the various dye stuffs. Large quantities are rarely taken by human beings, and it seemed of greater importance to obtain some idea of the toxic properties of the various groups than to determine exactly the lethal dose of each dye In some instances of course this was desirable, as in the case of commonly used colours, the composition of which might lead one to suspect that they were specially injurious, but as a rule such an investigation did not appear desirable. As will be shewn we have isolated observation on dyes of various classes by different experimenters, but no attempt has hitherto been made to select examples from each chemical group and submit them to careful tests. As will be pointed out in the part of this paper dealing with the methods employed, fish were used to enable one to judge accurate-

ly,if possible,of this relative toxicity. This was the chief reason for employing them,but it was also intend ed to make an effort to discover the effect of dyes when passed into streams. This very important subject has scarcely been approached by any observer. The evil results produced on river life by large quantities of sewage,by the effluents from breweries,paper works,and other forms of manufactories have been noted and reme-dies suggested,but the subject of dye impurities has met with scant recognition,and it is only of late that public attention has been turned to our many discolour-ed streams, in which the fish have been killed & which constitute an eyesore and a blot on the face of nature.

It must be remembered however that dye effluents contain many substances besides the mere colouring mat-ters and quite recently in the case of the river Leven in Dumbartonshire,an attempt was made to distinguish between the effects due to mordants and those caused by the dye stuffs. Some attention has also been direct-ed to this side issue in the following research.

The Artificial dyes are complex chemical bodies, and it is found that in many instances each of them has several commercial names,while the constitution of some of them has not yet been discovered. In noting the work done upon them in general,it will be well,as in the other cases,to classify them,considering only the most important groups.

The classification adopted by Weyl is here employed, as it is very convenient and he is an acknowledged authority on the subject.

For our purposes it may be termed Weyl's classification, although not really invented by him.

Weyl's Classification based

on Chemical Constitution.

1. Nitroso Colours.	Naphthol Green, B., Solid Green.
2. Nitro Colours.	Picric Acid, Martius' Yellow, Naphthol Yellow S., etc.
3. Azo Colours.	Anilin Yellow, Chrysoidine Bismarck Brown, Biebrich Scarlet, etc.
4. Triphenylmethane Colours (Anilines properly so-called).	Fuchsin, Malachite Green, etc.
5. Rosolic Acid Colours.	Corallin, Rosolic Acid.
6. Phthaleïn Colours.	Eosin, Erythrosin.
7. Anthracene Colours.	Alizarin, Alizarin Orange.
8. Indigo Colours.	Indigo.
9. Quinoline Colours.	Quinoline Yellow, Cyanine, Chrysaniline.
10. Indophenol Colours.	Methylene Blue.
11. Azine Colours.	Saffrinin, Magdala Red.
12. Aniline Black.	

1. Nitroso Colours.

Dinitroso resorcinol. Weyl experimented on dogs with this substances, and found that even large doses (one to two grams) administered by the stomach were non-toxic, while by hypodermic injection, in the proportion of .19 grams per kilogram of body weight, a

fatal result was obtained within 24 hours, the animal refusing food and becoming very weak. Post mortem congestions of various internal organs were found and an oedematous condition of the brain. (A table of Weyl's experiments will be found further on). This dye is chiefly used to dye cotton the dark green known as Resorcinol, Alsace, or solid green. No account is forthcoming of its effects upon workpeople in cotton factories.

Naphthol Green B. Weyl concluded from his experiments on dogs that this substance was quite harmless when administered by the stomach, but that hypodermic injection resulted in death due to abscess formation and septic fever.

Its commercial use is similar to dinitroso resorcinol, and no cases of poisoning in human beings are recorded as far as I know, while neither of these substances are likely to be used in the colouring of food stuffs or confections.

2. Nitro Colours.

They are chiefly orange or yellow coloured salts of the Nitro compounds, and with the exception of Picric acid, are largely employed in the coloura-

tion of food. They used to be largely employed in dyeing wool and silk, but are not very "fast" colours, and so are being replaced. The most important of the them is:-

Picric Acid. This acid was formerly largely used for dyeing silk, wool, or artificial flowers, and it has been employed in foods. It came, however, to be regarded as very poisonous, and in Germany its use as a food colour was forbidden in 1888. Its toxic properties, however, have probably been much exaggerated, as shewn by the experiments of Erb and Weyl. The former gave a rabbit weighing 1700 grams, ·06 gram of potassium picrate daily for ninety days. The only result was slight loss of weight and diarrhoea. A rabbit weighing 2065 grams died at the end of nineteen days, having been given 2·52 grams of the substance.

The latter experimented with dogs and found them fairly resistant to the action of sodium picrate in repeated doses, both by the stomach and hypodermically. Vomiting, diarrhoea, prostration and some dyspnoea, were the manifestations, but death only resulted after a large quantity had been taken. Rymsza of Dorpat has also devoted attention to this subject. Cases of poisoning in human beings are on record, both from picric acid and the picrates. Lewin found record of

three cases, and Adler has reported a case in a school girl, aged sixteen, who took from 3 to 5 grams of the acid. Vomiting and diarrhoea occurred promptly. Chlorosis ensued, and dark yellow staining of the skin, while, though the fingers of both hands were flexed at the metacarpo phalangeal articulations, the fingers themselves remained straight and rigid, and the patient had little power over them. The blood contained many white and few red corpuscles, and the acid was found in the urine. She recovered in about a week. These are said to be the usual symptoms, but according to Weyl, a healthy adult can bear from ·54 to ·90 grams potassium picrate in daily doses for a considerable time.

Di nitro cresol. This is also known as Saffron-substitute, Victoria Yellow, or Anilin Orange. Its salts have been used in the colouring of food and drink. They have been used for dyeing feathers, and coloured the hands of the women workers yellow, while they caused severe burning and itching. Later there was bleb formation and the blebs became confluent. The same condition occurred in the feet.

Weyl, from numerous experiments on rabbits and dogs, concludes that di nitro cresol is a very dangerous substance. He used samples from laboratories,

commercial samples, and a sample from a case of fatal poisoning in a woman who took about 4.5 grams as an abortifacient. He administered the substance both by the stomach and by hypodermic injection, and in nearly every case death ensued, the chief symptoms being vomiting, dyspnoea, tremors and spasm, severe cramps and tetanus, and great prostration. In the case of the woman mentioned above (Bremerhaven case) death ensued 5 hours after the powder had been swallowed. Vomiting was the chief symptom, and post-mortem, yellow staining of mucous membranes was found.

Martius Yellow, which is employed in printing on textiles, and for colouring Maccaroni, has also been found to be poisonous as shewn by the researches of Weyl, and those of Cazeneuve and Lépine. Haemorrhagic gastritis follows its administration, albuminuria and great dyspnoea are common symptoms and there can be no doubt it is an injurious colour.

Naphthol Yellow S. which has many other names is used in the printing of wool and silk and the colouring of food stuffs. Although only differing from Martius Yellow by the possession of one sulphonic group, and though it is more soluble, it is found to be harmless when given to dogs by the stomach or subcutaneously. Jacobs however found that its

prolonged use caused dermatitis.

Brilliant Yellow which also contains the sulphonic group is harmless to dogs even in large doses.

Aurantia or Imperial Yellow is said by some to be poisonous while others deny this. Certain samples produce vesicles on the skin, and a case is on record of skin affection following the use of gloves dyed by this substance. No definite experiments are forthcoming with regard to its action.

3. Azo Colours.

These as will be shown later constitute a very important group and their poisonous action has to some extent been investigated by Weyl and other observers. They contain a great number of colours which are chiefly used in the dyeing of textile fabrics, leather,etc. It is said however that Biebrich scarlet, a well-known member of the group has been used in Paris to convert ordinary oranges into blood oranges.

Azo Benzol a "parent" substance causes in dogs and rabbits bloody urine and vomiting (Baumann and Herter.).

Azo Oxy Benzol a similar substance is found according to Lewin to kill rabbits in from one to three

days, when given in doses of from .5 to 1 gram. Their livers were found to be enlarged, yellow and fatty, and the blood shewed methaemoglobin.

Turning to the dye stuffs themselves we find that Chrysoidine, Bismark Brown, and the Sodium salt of Amido azo benzol sulphonic acid produce eczema according to Blaschko and to Molènes and Lermoyez. Cazeneuve and Lépine have made some experiments and the following list embodies their conclusions. They worked with Mon azo colours.

Rouge Soluble, not poisonous to human beings.

Rouge Pourpre do. do.

Fast Red B. do. do.

Xylidin Red not poisonous to dogs either by stomach or subcutaneously.

Tropaeolin 000 do. do.

Solid Yellow do. do.
doubtful if poisonous to human beings

Weyl experimented with several of the Azo and Dis-azo groups, and concluded as follows:-

Mon-azo Group.

Non-poisonous. Bismark Brown, Soudan I, Ponceau 4 G.B., Archil substitute, Chrysoidine, Diphenylamine orange, Azarin S, M-nitrazotin, P-nitrazotin.

Poisonous. Orange II, Metanil Yellow.

Dis-azo Group.

Non-poisonous. Fast Brown G., Wool black, Naphthol black P., Congo Red, Azo blue, Chrysamine R.

Poisonous. Naphthol black P. had an injurious effect when given subcutaneously.

For a more detailed account of these researches, giving toxic doses, etc., see the appended list of Weyl's results, which I have tabulated.

Weyl sums up the results of his experiments as follows:-

"Of the twenty three azo colours subjected to examination only two, metanil yellow and orange II produce such effect when administered by the stomach, that we can consider them poisonous. With dogs the lethal dose is less than one gram per kilo. of the body weight of orange II, and only .53 gram per kilo. of metanil yellow. Of the remaining colours some produce vomiting (e.g. Bismark Brown), and others diarrhoea (Fast Brown, Chrysamin R), and many develop a slight albuminuria. The phenomena produced by subcutaneous administration are not all susceptible of the same interpretation. The abscesses were in some cases (for instance,Azo Blue) referable to the invasion of micro-organisms. Naphthol Black P. however is plainly poisonous when introduced into the subcu-

taneous cellular tissue. A striking fact is how long, in some cases, the aqueous solution of the colour remains unabsorbed in the subcutaneous cellular tissue. Congo Red, for instance, in experiment (iii) could be recognised in considerable amount seven days after the injection.

The urine obtained from the animals fed or treated subcutaneously with the azo colours was generally coloured and contained the unchanged colour only when considerable quantities of the material had been administered. A portion of the colour administered was found in the faeces and especially when the colour was insoluble.

Generally the complex colour molecule is split up in the animal organism into uncoloured derivatives. When animals, to which azo colours have been administered, have excreted a urine of normal colour, I have never been able to obtain any colouring matters analogous to the azo body used."

Few observations are forthcoming, and but little experimental work has been done, with regard to dyes belonging to the other groups. It is said that workers in Anilin colour works are apt to suffer from eczema, swellings of the face, vomiting, diarrhoea, and occasionally anaesthesia and paresis. They often

show indelible spots on the cornea, conjunctiva, head, breast, face and neck without general symptoms. Local changes of a more serious kind may be found in the skin and mucous membranes.

Continuing the consideration under Weyl's classification we find under

4. Triphenylmethane Colours, or Rosanilin Group, pure Fuchsin is non-injurious to men and animals, according to Bergeron, Clouet, Cazeneuve, Sonnenkalt and Bergmann. It very commonly however contains Arsenic, and workers with it have been found to suffer from phlegmonous abscesses (Rehn,). It has caused nephritis in dogs (Feltz and Ritter) and itching, colic and diarrhoea in man.

Arsenicated Fuchsin has been found in wines and confections.

Marron and Ceris behave in the same way, while Grenadine contains even more arsenic and is fatal to animals, producing diarrhoea and marasmus.

Water Blue and Split Blue, the latter a salt of triphenyl rosanilin, cause inflammation of the skin in those who work with them, Split Blue is injurious to rabbits.

Methyl Violet. This dye is a mixture of penta methyl and tetra methyl with some hexa methyl

para rosanilin, and Auramine produce skin inflammation, necrosis, pain, oedema, fever, vomiting, and headache. They have been given to rabbits without ill effect.

Malachite Green is only poisonous when impure.

Crystalline Green in one case, in workers long uninfluenced, caused inflammation of the skin of the hands and feet with the formation of blisters.

5. Rosolic Acid Colours.

They are non-poisonous. Small animals can bear one gram and more. In Austria however they are forbidden to be used as colouring matters for food stuffs

Corallin is often impure containing according to Guigot, Arsenic and Carbolic Acid. Jardine in 1869 demonstrated this dye to be harmless when pure

6. Phthaleïn Colours.

Eosin. Workers with this dye suffer from pain in the hands, hyperaesthesia and finger abscesses. These symptoms however are probably due to the strong chloride of lime they use to cleanse their hands.

Eosin and Erythrosine are said to be non-poisonous to rabbits even after prolonged administration. Lewin however regards both these and Phenol phthaleïn, when long continued, as injurious.

7. Anthracene Colours.

According to Ehrlich 1 c.c. of a saturated solu-

tion of Alizarin Blue S. injected subcutaneously into rabbits kills them within a quarter of an hour.

8. Indigo Colours.

Indigo Blue according to Weyl is non-toxic.

9. Quinoline Colours.

The effect of this group on the public health does not seem to have been noted.

10. Indo-Phenol Colours.

Methylene Blue after long continued administration of .5 to 1.5 gram has been found to produce irritation of the bladder and blue colouration of the urine and saliva. There was also headache, diarrhoea, muscular twitchings, giddiness and delirium.

11. Azine Colours.

Saffranin is said to be poisonous when injected intravenously. When given in powder form by the mouth, diarrhoea alone was produced.

12. Anilin Black.

This is very insoluble, and is always used along with many other substances. No record of its toxic properties is forthcoming.

METHOD OF INVESTIGATION.

This at first presented some difficulty. Weyl and previous observers experimented chiefly with dogs, but owing to the vomiting produced when the substances were given by the mouth, this method appears to have been unsatisfactory, and a comparison of the toxic effects of the different compounds impossible. Two of my experiments were made on dogs. One was the administration of Anilin Yellow (3 grms.) by the mouth. A few hours afterwards there was vomiting, and a large quantity of the dye stuff was rejected. The other was when the same quantity of Bismark Brown was given. Vomiting ensued in an hour and a half, and was frequently repeated, so that it was impossible to tell how much of the dye had been retained.

In order to compare the toxicity of the substances it was therefore necessary to use animals incapable of vomiting, and which at the same time were of such a size as to enable a fair amount of the dye to be given. For hypodermic medication small animals are to be preferred, as a much larger quantity can relatively be given them than can be given in

the case of large animals. For these modes of experimenting therefore, rabbits and rats, or guinea pigs were respectively used. As it is impossible, however, to be certain of the same degree of assimilation or absorption in all cases, a strictly comparative investigation of the various dyes could not be carried out. In most of the experiments the absorbability was proved by demonstrating the dye in the urine, but the rapidity of absorption, which is of primary importance, is not so easily demonstrated. In order therefore to carry out as completely as possible a comparison of the toxicity of the various dyes, fish have been used. These could be more easily controlled, and the experiment carried out in a more exact manner. The experiments themselves were of the simplest. A definite amount of the dye was dissolved, or in a few cases, partially dissolved in a definite quantity of water, usually 5 litres. The fish were placed in the solution in which they could freely swim. They were watched, and the lethal period noted. It was found by control experiments that the fish employed remained well and lively for twenty-four hours, and even much longer periods, in a similar or smaller quantity of pure water. In a few cases after death a diffuse stain-

ing was noticed, affecting specially the nervous system, but microscopic examination of the tissues failed to shew anything of interest. This mode of experimenting is of importance in connection with the pollution of streams by mill and factory effluents, which was one of the primary objects of this investigation. Not only were solutions of dyes and mordants thus tested, but samples of effluents and of water from polluted streams and rivers were obtained and their effects on fish duly noted. Roach, dace, and gudgeon were used, as these were the only species which could readily be obtained at all seasons. Some of the dyes were mixed with fluff or impurities which remained suspended or formed deposits. Others again formed curious suspensions or scums upon the surface, and when this occurred it will be found mentioned; though, with the exception of one or two cases, it looked as if death was never due to the mechanical action of the suspended matter which indeed seemed rarely to have any effect.

In the case of rabbits, the dye was administered in "single dose" experiments, in solution or suspended, by the oesophageal tube; in continued experiments, the colour was mixed with the food (crushed oats or oatmeal porridge). In the case of rats and

frogs, a solution was injected hypodermically.

Choice of Dyes.

In the choice of dyes used, more attention was paid, as far as this was possible, to their chemical constitution than to their commercial value. For it is obvious that if we can determine the effect of primary substances, the change these undergo when combined with various radicles, the effect of the radicles upon each other, etc., we shall have the means of indicating the probable toxicity of substances not investigated. The commercial aspect, however, has not been neglected, and the dyes in common use, if not otherwise contra-indicated, have been used. Thus the Azo dyes, which are now most extensively employed, have received special attention. The classification adopted was that used by Nietzki, which is practically that employed by Weyl, though the sub-divisions are better marked. It is as follows:-

Group.	Dyes used.
1. Nitroso	Naphthol Green B.
2. Nitro	Picric Acid. Martius Yellow. Naphthol Yellow S. Aurantia. Poirrier's Orange.

Group		Dyes used.
3. Azo.		Anilin Yellow. Chrysoidine. Bismark Brown. Brilliant Orange. Tropaeolin 000 No.2. Orange G. Orange III. Orange G.T. Xylidene Red. Cumidene Red. Fast Red B. Biebrich Scarlet. Brilliant Croceïn. Helianthin. Tropaeolin 00. Congo Red. Hessian Purple. Primuline. Rosazurine G. Heliotrope Ponceau R.R. Azo phenyl Blue.
4. Triphenyl methane	Rosanilin	Malachite Green. Rosanilin. Methyl Green. Rosanilin Acetate. Rosanilin Blue. China Blue. Alkali Blue. Hofmann's Violet.
	Rosolic Acid	Rosolic Acid. Aurin.
	Phthaleïn	Fluoresceïn. Eosin (Yellow shade).
5. Oxyquinone		Alizarin. Purpurin.
6. Ketoneimide		Auramine
7. Quinone-imide	Indophenol	Methylene Blue.
	Oxyindamine	Gallocyanin.
8. Indigo		Indigo carmine
9. Quinoline		Phosphine.
10. Azine		Saffranin. New Blue.
11. Indulin		Nigrosin.

As will be seen from each of the groups, one or more dye has been taken, most when the colours were

of considerable importance, least when they had fallen into disuse. In the Azo group some attempt has been made to modify the simpler compounds by the introduction of various radicles, and it is in this group principally that, owing to its commercial importance, an effort to discover some rule controlling the reaction between chemical constitution and toxicological effect has been made.

Most of the dyes were obtained from Merck of Darmstadt, and appeared to be pure. Where there was any doubt as to this a chemical or microscopical examination was made, special care being taken to eliminate any chance of Arsenic being present in the case of the green dyes. Commercially, dyes are standardized to a definite colour by the addition of dextrin, salt, or sodium sulphate. These substances were not found in the dyes employed.

As already stated some experiments were made with mordants on fish. This was rendered necessary as some of the dyes require mordants to fix them, and the effluents contain both dyes and mordants. In the case of the effluents and river waters examined, it was impossible to determine quantitatively their composition; but substances which have been in the liquid at one or other time are given.

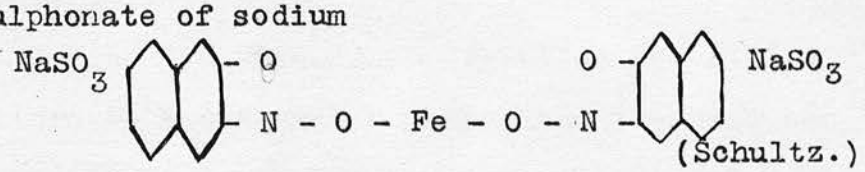
These experiments will be mentioned and described in their appropriate places.

Experiments.

I. Nitroso Group.

The substances of this group form fairly stable colouring matters. They are produced by the action of nitrous acid on certain of the phenol derivatives and their sulphonic acids and form salt-like combinations with iron, nickel, and cobalt. They probably contain the dyad iso nitroso group, N O H, and according to Weyl are preferably called iso nitroso colours. They are used for the dyeing of wool, silk, and cotton. The colour is either produced upon the fibre, or the fibre is impregnated with a ready formed colour, as in the case of the example used, namely

1. Naphthol Green B. Ferrous nitroso naphthol sulphonate of sodium



(Schultz.)

is a dark green powder, neutral in reaction, and fairly readily soluble in water. Roach in 1 in 1000 solutions remained unaffected after 24 hrs.

Want of material prevented further research with this dye.

II. Nitro Group.

The nitro colours contain the group NO_2 and are produced by the action of concentrated nitric acid on the hydrocarbons and their derivatives. They are mostly orange or yellow coloured salts, and are not so much employed as used to be the case. The most important:-

1. Picric Acid. Trinitrophenol

$$C_6 H_2(NO_2)_3 OH$$

is prepared on a large scale by the action of strong nitric acid on phenol sulphonic acid. It is a pale yellow, crystalline powder, and was once used largely in wool and silk dyeing. It is a powerful substantive colour. It has been added to beer to give it a bitter taste, and as already stated, has been subjected to much investigation.

a. Roach in 1 in 10,000 solution was killed within 24 hours, which means that the fish was alive 8 hours after insertion and succumbed during the night. It was slightly stained externally.

Roach in 1 in 100,000 solution was not affected in 24 hours. Injection into rats was unsatisfactory, owing to comparative insolubility.

2. Martius Yellow. Di Nitro - a - Naphthol.

$$HO(NO_2)_2\ C_{10}H_5$$

is an orange yellow powder, rather insoluble in water. Occurs in dye form as the Calcium or Soda salt. It is used in wool and silk dyeing and has also been employed to colour confectionery, as it has not the bitter taste which most nitro-compounds have.

Gudgeon in a	suspension	of	1	in 10,000	died in 1 hour.
" "	solution	"	1	" 100,000	" within 24 hrs
" "	"	"	1	"1,000,000	remained unaffected for 24 hours.

There was no staining produced in any case.

3. Naphthol Yellow S. Sodium di nitro - a - naphthol sulphonate $C_{10}H_4HO(NO_2)_2NaSO_3$

is a sulphonated Martius Yellow, and is also used for wool and silk.

Gudgeon in a solution of 1 in 5000 remained unaffected for 24 hours, even though it was in a weak and fungoid condition.

4. Aurantia. Ammonium salt of hexa nitro di-

phenylamine. $NH_4N\left[C_6H_2(NO_2)_3\right]_2$ is a yellow powder, not very soluble in water, and used for the orange dyeing of wool, silk and leather, though the Azo dyes have largely replaced it. The sodium salt was used.

a. Roach in solution of 1 in 5,000 (slight suspension) died in 1½ hours.
" " " " 1 " 10,000 " " 2 "
" " " " 1 "100,000 " " 6 "
" very pale co- "lored solution 1 "500,000 " "24 "

In all cases there was well marked spasm and twitchings, while, besides complete external staining, the tissues - especially the brain and spinal cord - were found stained. Microscopically the staining was found to be diffuse, and quite useless from an histological point of view.

b. A rabbit weighing 1 k. 620 grm. was given 4.860 gr. of this colour, or 3 grm. per kilo. at 12.15 p.m. There was no effect till the second night when appetite seemed to be impaired. The animal was found dead on the morning of the third day.

Post mortem. The mucous membrane of the stomach was found stained in parts, and shewed a

few minute haemorrhages. There was some congestion of the large intestine. The kidneys were congested, and the urine was found to contain albumin and casts. The brain and spinal cord were not stained, and appeared normal.

5. Poirrier's Orange Meta nitranilin B naphthol di sulphonic acid, is an orange coloured powder, solutions of which gave a neutral reaction.

a. Roach in solution of 1 in 5,000 died in 4 hours
" " " " 1 " 10,000 " " ? "
" " " " 1 " 100,000 remained unaffected for 24 hours.

There was slight external staining produced.

III. Azo Group.

This at present is the most important, as it contains a great number of useful dye stuffs which have largely replaced many of the formerly used colouring matters. As most attention has been paid to them in this paper, it seems but fitting to enter into a rather more detailed account of their chemistry, though it will be made as short and practical as possible.

The Azo dyes are hydroxyl or amido deriva-

tives of the simple azo compounds, which in their turn are derived from the mother substance Benzene C_6H_6. In the azo compounds the diatomic group and chromophor - N:N- is introduced, and it is bound on either side to a carbon atom. The simple azo bodies, such as Azo benzene = C_6H_5-N:N - C_6H_5, are usually brilliantly coloured, but they are not dyes, as they do not possess the property of combining with either acids or bases.

The azo colouring matters can be obtained from these simple azo compounds, but are more usually prepared by the action of the unstable diazo compounds on phenols (OH group) or amines. (NH_2 group). It is an easy and ready method, while a variety of combinations can also be made The following reaction is an example of the preparation of an azo dye stuff from the action of a diazo compound on a primary amine.

(1) C_6H_5-N:N-Cl. + $C_6H_5NH_2$ = C_6H_5-N:N-NH·C_6H_5+HCl.

Diazo benzene chloride — Anilin — Diazo amido benzene

(2) C_6H_5-N:N-NH·C_6H_5 = C_6H_5-N:N-$C_6H_4NH_2$ } Anilin Yellow.

Diazo amido benzene — Amido azo benzene

It is thus seen that the introduction of salt forming (auxochromic) groups is necessary,

if we are to get the dye stuffs, and the stability of the dye is found to depend on the number of the salt forming groups present. All Diazo compounds + Amines give yellow, red, or brown dyes, but not blue or violet. The scarlet dyes, however, are the most important and comprise the greater number of the azo dye stuffs used in commerce. Substituted products or homologues of di azo benzene chloride on primary, secondary, or tertiary Amines give azo dyes.

Of these dyes the Sulpho acids are chiefly used in commerce, because their Soda salts are soluble in water. They are obtained by the action of sulphuric acid on the dye stuff.

Thus if Chrysoidine = di amido azo benzene is treated with sulphuric acid, its sulphonic acid is obtained.

<u>Reaction</u> = $C_6H_5-N:N-C_6H_3(NH_2)_2 + H_2SO_4 =$

$C_6H_5-N:N-C_6H_2\begin{matrix}HSO_3\\(NH_2)_2\end{matrix} + H_2O$

In the following experiments it will be found that in the case of rabbits, the so-called "parent" substances, azo benzene and azo toluene, were first administered. Also that "single dose" and "long continued" experiments were performed. The effect of azo benzene on fish could not be

tested owing to its insolubility.

Azo benzene $C_6H_5-N:N-C_6H_5$
is a bright red crystalline substance obtained by the reduction of nitro benzene. The following are the full records of the three experiments with it upon rabbits, the symptoms being carefully noted, the rectal temperature, the pulse, and respirations being frequently taken, and a post mortem being performed in the two fatal cases.

1. Jan. 25th 1897. Rabbit. Weight 1,480 grms.

3·30 p.m. Pulse 168. Respirations 96. Temperature 38·4°C. Condition, Ear vessels somewhat dilated. Otherwise animal normal.

3·40 p.m. ·37 grms.=¼ grm. per kilo body weight injected into stomach suspended in water and mucilage.

4 p.m. P. 192. R.108. T. 38°C. No change apparently.

4·15 p.m. As above (at 4) Do. Do.

4·50 p.m. T. 37·2°C. No other change.

Jan. 26th.

11·35 a.m. Rabbit seems well and lively. No special change except some yellowness of conjunctivae. P. 204. R. 72.

T. 37·3°C. Only a few c.cs of urine were passed during the night. The faeces appeared perfectly normal.

5·20 p.m. No special change. T. 38°C. Much faeces passed.

Jan. 27th.

10 a.m. Rabbit as before. Conjunctivae still yellowish. P. 204. R. 96. T. 36·6°C.

Jan. 28th.

Rabbit well. Urine and ethereal extract of urine examined and nothing abnormal found. Animal continued healthy.

2. Jan. 29th 1897. Rabbit. Weight 1,590 grms.

3·40 p.m. P. 192. R. 96. T. 38·3°C. Normal.

3·45 p.m. ·8 grms. = ½ grm. per kilo. body weight injected as above.

4 p.m. P. 204. R. 84. T. 38·3°C. No change.

4·45 p.m. Slight fall in temperature. No other change.

5 p.m. No change.

Jan. 30th.

Rabbit looked ill. Marked yellowish brown hue of conjunctivae. Ear vessels contracted. P. 180. R. 96.

T. 37·5°C. No other change. No urine passed during the night.

Jan. 31st. (Sunday).

Rabbit found dead. Died about 11 a.m.

Feb. 1st.

Post mortem. Food in stomach. No abnormality of mucous membrane of stomach. No duodenitis. One Peyer's patch found congested. Venous congestion of spleen present. Kidneys congested and microscopically shewed acute catarrhal and haemorrhagic nephritis. Bladder, which was not affected, contained 20 c.c. of bloody urine. Spectrum of the blood did not shew methaemoglobin, and azo benzene added to normal blood produced no appearance of methaemoglobin.

3. Nov. 19th 1896. Rabbit. Weight 2,150 grms.

4 p.m. P. 180. R. 108. T. 38·3°C.

Condition. Reflexes and pupils normal. Conjunctivae rather pale. Ear vessels somewhat contracted.

4.20 p.m. 1·65 grm. = about ¾ grm. per kilo. body

weight injected into stomach as above.

4·25 p.m. & at intervals to 4:45. P. 180. R. 96 to 120. No change.

4·50 p.m. T. 38°C. Reflexes and pupils normal.

5 to 5·25 Conditions remained the same.

Nov. 20th.

10 a.m. P. 168. R. 96. T. 35°C. Reflexes normal and ear vessels unchanged, but rabbit looked heavy and ate sluggishly with eyes half closed. Conjunctivae were very pale with a brownish tinge. Brownish green urine (20 c.c.), opaque and odourless, passed during the night. Ethereal extract made of it, and this on microscopic examination shewed sheaves of yellow acicular crystals in double rosettes.

3·35 p.m. Pulse feeble. R. 96. T. 32·8°C. Rabbit weaker. Ears drooped more. General appearance of methaemoglobin. Reflexes present but more sluggish. No change in ear vessels.

4·15 p.m. T. 31·4°C.

4·25 p.m. Rabbit in clonic convulsions. Marked muscular weakness. Pulse 180 and very

weak. R. 168. Pupils a little dilated.

4·35 p.m. Tonic convulsions lasting a few seconds T. 30·6°C. Conjunctival and nose reflexes still present. No urine or faeces passed during the day.

4·45 p.m. Death.

5 p.m. Post mortem. No abnormal appearance in stomach which was much distended with food. Some haemorrhagic duodenitis present. Haemorrhagic inflammation of small intestine to within 15 inches of the Caecum. Small haemorrhage in pylorus of right kidney which shewed acute, catarrhal, haemorrhagic nephritis. Bladder empty. Uterus and thoracic organs healthy. Microscopic examination of duodenum and small intestine shewed great extravasation of leucocytes and congestion. Spectroscopic examination of blood shewed no evidence of methaemoglobin.

Azo Toluene. $C_6H_5-N:N-C_6H_4(CH_3)$

is a mono methyl derivative of azo benzene which

it resembles.

4. Jan. 12th 1897. Rabbit. Weight 1,690 grm.

11.50 a.m. Condition normal. P. 192. R. 84.
T. 38·2°C.

12·15 p.m. ·38 grm. injected as above.

12.30 p.m. No change. till

3 p.m. when rabbit looked ill. . P. 204. R. 60
T. 38·5°C. No appearance of methaemoglobin. No further change that day.

Jan. 13th.

11·15 a.m. Rabbit had eaten very little and looked seedy. The pulse was feeble, but there was no other change. P. 204. R. 72. T. 38·5°C. About 35 c.c. of brownish green urine passed during night. Reaction slightly alkaline. No albumin. Microscopic examination shewed nothing abnormal. No change till

5 p.m. when animal began to improve and become livelier. T. 39°C.

Jan. 14th.

12 a.m. Rabbit quite well. Urine again examined, also ethereal extract with entirely negative results as regards abnormality.

IV. Azo Colours.

1. Anilin Yellow - Amido Azo benzene.

$$C_6H_5\text{-}N\text{:}N\text{-}C_6H_4NH_2$$

is a reddish yellow powder, rather sparingly soluble in water, forming a fine red coloured solution. It comes into commerce as the hydrochloride, but is not much used as a dye, being far from fast and volatilising easily on steaming.

a. Roach and gudgeon remained well and lively for 24 hours in 1 in 1000 solutions, the only result being some external staining. Same result was obtained with solutions 1 in 500 and 1 in 200.

b. Nov. 25th 1896. Rabbit. Weight 2,690 grm.

4·30 p.m. Animal normal. Ear vessels slightly dilated. P. 204. R. 132. T. 37°C.

4·35 p.m. ·68 grm. injected into stomach as in previous cases.

4·40 p.m. and at intervals to 5·30. No change beyond slight fluctuations in temperature.

Nov. 26th.

11 a.m. Rabbit quite well. No change. P. 180. R. 144. T. 37·8°C. Urine

and faeces passed during night, but owing to mistake were not collected.

3·30 p.m. Conjunctivae considerably paler. Pupils dilated. Ear vessels rather distended. Reflexes normal. Animal running about briskly. No further change that day, or on succeeding days. Urine examined. Greenish brown in colour, contained the dye, as shewn by chemical test with sulphuric acid. No albumin.

c. Feb. 5th to March 8th. Rabbit was given daily in food 1·14 grm.- ½ grm. per kilo. body weight. Animal increased in weight and was wholly unaffected. Urine was frequently examined and found to contain the dye, but no albumin.

d. July 30th. in the morning a Dog weighing 5,800 grm. was given in food 3 grm. Vomited in the afternoon. No effect.

e. 1 c.c. of 1% solution injected subcutaneously into a Frog weighing 29 grm. No immediate or after effects. Dye present in the urine.

2. Chrysoidine - Di amido azo benzene.

$$C_6H_5\text{-}N{:}N\text{-}C_6H_3(NH_2)_2$$

occurs in commerce as the hydrochloride in the form of minute, dark violet crystals having a metallic reflex, and producing a rich yellow solution. It is chiefly used in cotton dyeing being mordanted on the fibre with tannic acid.

a. Roach and Gudgeon were killed in from 10 to 24 hours by a solution of 1 in 200,000; in from 4 to 6 hours by 1 in 100,000; and in a few minutes by solutions of 1 in 10,000, and 1 in 1000. All the fish were brilliantly stained externally, and also shewed deep staining of the brain and spinal cord, and a well-marked staining of muscles, alimentary canal, &c. This remarkable result induced one to examine the tissues microscopically, but the staining was found to be diffuse. The colour seems to pass into the blood stream circulating through the gill arches, as dead fish placed in strong solutions shewed no internal staining, though they were deeply stained externally.

b. Nov. 26th 1896. Rabbit. Weight 1,650 grm.
4 p.m. Condition normal. P. 192. R. 144.
T. 38°C.

4·30 p.m. 3 grm. injected as usual.

4.50 p.m. and at intervals to 5·15. No change observed.

Nov. 27th.

9·15 a.m. P. 192. R. 144. T. 39·3°C. No change. Urine and faeces passed during night. Animal remained well during this and succeeding days The urine, which was examined on two occasions was of a dark orange colour, contained the dye, but no albumin or casts.

c. 4 grm.= 2 grm. per kilo. body weight were given daily in food to a Rabbit weighing 2 kilos. from March 2nd to 29th, when, for lack of material, experiment was discontinued. Absolutely no effect was produced, there being no albumin in the urine.

d. In order to see if the internal organs were stained, a guinea pig was given this dye in the food. It received altogether 3½ grm. and was killed on the third day.

Post mortem. Mucous membrane of mouth and tongue stained yellow. No staining of internal organs. Kidneys examined microscopically, but no appearance of dye stuff found in tubules or glomeruli. No nephritis.

e. Rat received by subcutaneous injection, with antiseptic precautions, ·1 grm. dissolved in distilled water. No effect.

f. 1 c.c. of saturated solution injected subcutaneously into Frog (30 grm) No immediate or after effects.

3. Bismark Brown - Tri amido azo benzene.

$$C_6H_5\text{-}N{:}N\text{-}C_6H_2(NH_2)_3$$

is a dark, brown powder, forming an orange coloured solution. It occurs in commerce as the hydrochloride and is employed in the dyeing of wool, leather, and jute, and is also mordanted on cotton with tannic acid, tartar emetic, or turkey red oil.

a. Roach and Gudgeon were killed by solution of 1 in 10,000 in 30 minutes; of 1 in 100,000 in

45 to 60 min., 1 in 200,000, and more dilute solutions had no effect. Staining was the same as in the case of Chrysoidine, but the internal staining was not so marked.

b. Dec. 1st 1896. Rabbit. Weight 1,760 grm.

3·15 p.m. Condition normal. P. 180. R. 96. T. 38·7°C.

3·50 p.m. ·88 grm. = ½ grm. per kilo. body weight injected as usual.

4 p.m. P. 180. R. 120. T. 38·2°C. No other change.

4·10 p.m. and at intervals to 5·30. No change. No sign of illness.

Dec. 2nd.

10·10 a.m. P. 180. R. 96. T. 38·6°C. Rabbit well and lively. No urine passed during the night.

10·25 a.m. 20 c.c. of dark, brownish orange urine passed. Reaction acid. Dye present. No casts or albumin. Animal continued well the rest of that day and succeeding days.

c. 4 grm. = about 2 grm. per kilo. body weight given daily in food to a Rab-

bit weighing 2,300 grm. from March 10th to March 29th, without the least effect. Animal indeed increased in weight.

d. Dog weighing 5,800 grm. received on August 2nd 1897 at 11 a.m., 3 grm. Given in food. Dog vomited at 1.30 p.m. and frequently during the afternoon, and for several days looked ill and refused food. Dye was found in the urine.

e 1 c.c. of 1% solution injected into Frog weighing 40 grm. No immediate or after effects. Dye present in the urine.

Crocein &

4. Brilliant Orange Benzene azo B naphthol sulphonate of sodium.

$$C_6H_5\text{-}N{:}N\text{-}C_{10}H_5\begin{cases}OH\\SO_3NA\end{cases}$$

is a reddish yellow powder, dissolving not very easily in water to a red coloured solution. It is chiefly used in wool dyeing.

a. Roach & Dace were killed by a fine suspension of 1 in 5,000 in 5 hrs.
Do. Do. Do. a fairly clear solution of 1 in 10,000 in 6 hrs.
Do. Do. Do. a clear solution of 1 in 20,000 within 24hr.

A solution of 1 in 30,000 had no effect in

24 hours. There was external but no internal staining. Fatal cases shewed marked congestion of the gills, which were coated with mucus.

b. 4.8 grm.= 3 grm. per kilo. body weight were given daily in food to Rabbit weighing 1,600 grm. from July 12th to July 25th. No effect was produced save a diminution in the urinary excretion, sometimes only 10 c.c. being passed in the 24 hours. Urine and faeces were examined on several occasions and both found to contain the dye stuff. On the 17th and 18th July, albumin was present in the urine, but was merely temporary and was not again found.

c. 1 c.c. saturated solution injected subcutaneously into a Frog (34 grm.). No immediate or after effects. Frog chloroformed on following day.

Post mortem. Skin stained, owing to dye present in urine. No staining of nervous tissues. Dye found in the intestine.

5. Tropaeolin 000 No. 2. Benzene sulphonate of sodium azo B naphthol.

$$C_6H_4\begin{cases}SO_3Na\\N{:}N\text{-}C_{10}H_5\begin{cases}OH\\OH\end{cases}\end{cases}$$

is an orange red powder fairly easily soluble in water, forming a red solution. It is used for dyeing wool and silk.

a. Roach was killed in a fine suspension of 1 in 5,000 in 24 hours. A fairly clear solution of 1 in 10,000 had no effect in 24hr. There was external, but no internal staining.

b. 2 grm.= 1 grm. per kilo. body weight, were given daily in food to Rabbit weighing 1,850 grm. from Feb. 15th to March 8th. No effect on health or appetite was observed, though urine was coloured red, and contained the dye stuff. There was indeed slight increase in weight.

c. 1 c.c. of saturated solution injected subcutaneously into Frog.
No immediate or after effects.

6. Orange G. Benzene azo B naphthol di sulphonate of sodium

$$C_6H_5\text{-}N{:}N\text{-}C_{10}H_4\begin{cases}OH\\(SO_3Na)_2\end{cases}$$

is a yellowish orange powder fairly easily soluble in water.

a. Roach and Gudgeon were killed by a clear solution of 1 in 1,000 in 3 hours.
No effect was produced in 24 hours by a solution of 1 in 5,000, but the fish (gudgeon) died on the following day, after shewing signs of weakness and a fungus growth on the snout.
In both cases there was external but no internal staining, and in the first the gills were coated with a thick, coloured mucus.

b. 4.5 grm.= 3 grm. per kilo. body weight were given daily in food to a Rabbit, weighing 1,500 grm. from July 12th to July 26th.
No effect of any kind was produced. Urine and faeces contained the dye stuff.

c. 1 c.c. of 1% solution injected subcutaneously into Frog (28 grm.).
No immediate or after effects.

7. Orange III. Nitro Benzene azo B naphthol disulphonate of sodium.

$$C_6H_4\begin{cases}NO_2\\ N{:}N.C_{10}H_4\end{cases}\begin{cases}OH\\ (SO_3Na)_2\end{cases}$$

is a yellowish orange powder and is slightly used in the dyeing of wool and silk. It is not very soluble in water.

a. Roach in a fine suspension of 1 in 5,000 was killed in 5 hrs. Immediately on insertion there were signs of respiratory difficulty, the suspended matter clogging the gills. There was no staining.

b. 3.6 grm.= 3 grm. per kilo. body weight given daily in food to a Rabbit weighing 1,200 grm. from July 12th to July 19th, when experiment was stopped owing to animal refusing to eat the coloured oats. Animal began to look ill and lose appetite in two days. There was no staining of conjunctivae. The experiment was unsatisfactory. Dye was present in the urine. This experiment was repeated, 5 grm.= 3 grm. per kilo. being given in the same way to a Rabbit weighing 1,630 grm. from July 28th to

Aug.5. Animal took food well till August 2nd and remained unaffected, after which date there was loss of appetite for the coloured food, and rapid loss of weight. On August 5th Rabbit weighed 1,150 grm, and experiment having become unsatisfactory was discontinued.

8. Orange G. T. Tolueno azo B naphthol sulphonate of sodium.

$$C_6H_4\begin{cases}CH_3\\ N{:}N\text{-}C_{10}H_5\end{cases}\begin{cases}OH\\ SO_3Na\end{cases}$$

is a yellowish orange powder, rather insoluble in water. It is not of much commercial importance, but was here introduced as it contains the methyl group. (CH_3) constituting a Toluene compound.

a. Roach and Dace were killed in a turbid solution of 1 in 5,000 in from 12 to 24 hours. A clear solution of 1 in 25,000 produced no effect in 24 hours. There was slight external, but no internal staining. The gills were congested in the fatal case.
A fresh solution of 1 in 25,000 was also made every day from August 2nd to August 9th, and a roach thus kept constantly in the solution.

The fish remained well and lively, and there was no effect beyond slight external staining.

b. 3 grm.= 3 grm. per kilo. body weight were given daily in food to a Rabbit weighing 1 kilo. from July 23rd to August 3rd. Animal began to look ill on July 28th. Weight=970gr. The day following began to refuse the coloured food. On July 30th took all the food, which was in the form of a coarse porridge. After this animal became sluggish in its movements and at last wholly refused food. It was chloroformed on Aug. 4th. Urine contained the dye, but no albumin.
Post mortem. No staining of muscles, peritoneum, or brain. Stomach and intestines contained the dye. Microscopically kidneys shewed condition of early cloudy swelling. Exposure to air produced no effect on the kidney section which seemed to be faintly stained.

c. 1 c.c. saturated solution injected subcutaneously into Frog (28 grm.).
No immediate or after effects.

9. Xylidene Red. Xylene azo B naphthol disulphonate of sodium.

$$C_6H_3\begin{cases}(CH_3)_2\\ N{:}N\text{-}C_{10}H_4\begin{cases}OH\\ (SO_3Na)_2\end{cases}\end{cases}$$

is a bright red powder, fairly readily soluble in water, producing a fine red solution.

a. Roach was killed in a clear solution of 1 in 1,000, with a slight fluffy deposit, in 24 hrs. A perfectly clear solution of 1 in 5,000 had no effect in 24 hours.
There was external but no internal staining.

b. 3 grm.= about 3 grm. per kilo. body weight were given daily in food to a Rabbit, weighing 950 grm., from July 23rd to August 4th. Dye was present in the urine on July 24th. On July 29th weight was 810 grm., but there was no sign of illness till July 31st when diarrhoea set in, and this was followed by loss of appetite and rapid wasting. Weight on August 4th = 650 grm. Animal was found dead in its cage on August 5th.
Post mortem. Dye found in small intestine. Liver looked congested, being very dark in colour. Kidneys were swollen and translu-

cent looking. Microscopically they shewed cloudy swelling. Urine found in the bladder, and for the first time shewed the presence of albumin. Microscopically the liver exhibited nothing of importance, and exposure to air produced no effect on a thin section of the organ.

c. 1 c.c. of 1% solution injected subcutaneously into Frog (27 grm.).
No immediate or after effects.

10. Cumidine Red. Cumene azo B naphthol disulphonate of sodium.

$$C_6H_4\begin{cases}CH(CH_3)_2\\ N:N-C_{10}H_4\end{cases}\begin{cases}OH\\ (SO_3Na)_2\end{cases}$$

has the same characteristics as Xylidene Red, but is slightly more soluble in water.

a. Roach in solutions of 1 in 1,000 and 1 in 5,000 remained wholly unaffected during 24 hours. There was slight external staining, and stained scales were afterwards shed, fish dying in 4 days though in pure water.

b. 7 grm.= 3 grm. per kilo. body weight given daily in food to a Rabbit, weighing 2,300grm. from July 26th to August 3rd. There was no effect, though dye was present in the urine, and food was taken freely till August 1st. Thereafter animal shewed signs of weakness and refused food. Experiment was therefore stopped on August 3rd, the animal's weight being 1,950 grm.

c. 1 c.c. of 1% solution injected subcutaneously into a Frog (27 grm.).
No immediate or after effects.

11. Fast Red B. A naphthalene azo B naphthol disulphonate of sodium.

$$C_{10}H_7 -N:N-C_{10}H_4\begin{cases}OH\\(SO_3Na)_2\end{cases}$$

is a dark red powder, neutral in reaction, and forming a very deep red solution.

a. Roach was killed by a solution of 1 in 1,000, which contained a little suspended matter, in from 12 to 24 hours.
A solution of 1 in 5,000, dark and clear, had no effect in 24 hours, but fish, though af-

terwards kept in pure water, died in 7 days with loss of stained scales and marked fungus growth.

b. 4.7 grm.= 3 grm. per kilo. body weight was given daily in food to a Rabbit weighing 1,550 grm. from July 26th to August 3rd. Dye was present in urine, but there was no albumin, and food was well taken for 8 days, after which animal refused food and looked ill. Experiment was therefore discontinued on August 4th, animal then weighing 1,350 grm.

c. 1 c.c. of 1% solution injected subcutaneously into Frog (20 grm.).
No immediate or after effects.

12. Biebrich Scarlet. Benzene sulphonate of sodium azo benzene sulphonate of sodium azo B naphthol.

$$C_6H_4\begin{cases}SO_3Na \\ N{:}N\text{-}C_6H_3\end{cases}\begin{cases}SO_3Na \\ N{:}N\text{-}C_{10}H_6\ OH\end{cases}$$

is a fine red powder, used in wool and silk dyeing, and as already mentioned it has been employed to convert ordinary oranges into blood oranges.

The sample obtained was neutral in reaction.

a. Roach in a very turbid solution of 1 in 5,000 remained alive for 24 hours, but lost its tail, the stump of which was deeply stained. A solution of 1 in 10,000 in which there was a fine suspension did not affect a roach within 24 hours. Tail alone deeply stained. A fresh solution of 1 in 5,000 was also made every day from August 10th to August 19th and a roach thus kept constantly in the solution. The fish remained well and lively and there was no effect beyond a slight external staining. At the conclusion of the experiment the fish was killed, but no internal staining was found.

b. 1 c.c. saturated solution injected subcutaneously into Frog.
No immediate or after affects.

13. <u>Brilliant Crocein.</u> Benzene azo benzene azo B naphthol disulphonate of sodium.

$$C_6H_5\text{-}N{:}N\text{-}C_6H_4\text{-}N{:}N\text{-}C_{10}H_4\begin{cases}OH\\(SO_3Na)_2\end{cases}$$

is a bright red powder fairly soluble in water, & neutral in reaction.

a. Roach in clear solution of 1 in 500 was killed in from 12 to 24 hours.
Roach in a clear solution of 1 in 1,000 remained unaffected during 24 hours, but in a second experiment this solution proved fatal to a fish with roe in 24 hours.
In the fatal cases there was bright red external staining, while there was slight yellow internal staining, affecting especially the brain and the spinal cord.

b. Feb. 4th. 1897. Rabbit. Weight 1,510 grm.
3.20p.m. Condition normal. P.192.R.96.T.38.4°C.
3.25p.m. 3 grm.= 2 grm. per kilo. body weight, injected into stomach suspended in water and mucilage.
Observations taken at intervals till 4,30p.m. but no change observed.
Feb.5th 10 a.m. Rabbit lively and well. No change on this or succeeding days. Dye present in the urine which was of a fine red colour. No blood or albumin in the urine, and nothing abnormal found microscopically.

c. 4 grm.= 2 grm. per kilo. body weight given daily in food to a Rabbit weighing 2 kilo. from March 10th to March 27th. Absolutely no effect was produced, animal taking food freely, and remaining well and lively. It gained in weight somewhat.

d. 1 grm. dissolved in distilled water was injected, with antiseptic precautions, subcutaneously into back of Rat.
There was neither local nor general effect.

e. 1 c.c. of 1% solution injected subcutaneously into a Frog.
No immediate or after effects.

14. <u>Helianthin.</u> Benzene sulphonate of sodium azo di methylanilin.

$$C_6H_4\begin{cases}SO_3Na \\ N{:}N\text{-}C_6H_4N(CH_3)_2\end{cases}$$

is an orange yellow powder, but slightly soluble in cold water. . It is used in the dyeing of wool and silk, an acid bath being used in the process.

a. Roach was killed by a turbid solution of

1 in 5,000 in 24 hours.
A similar solution of 1 in 10,000 did not affect roach within 24 hours. In both cases there was slight external staining. No internal staining in fatal case, in which a scum formed on the surface.

b. Dec. 3rd 1896 Rabbit. Weight 1,470 grm.
5.p.m. Condition normal. P.192. R.120. T.38.2°C.
5.10. .74 grm. = ½ grm. per kilo. body weight injected into stomach in usual manner. Frequent observations made till 6.10 and no change observed.
Dec.4th. 10 a.m. Rabbit quite well. No change on this or succeeding days. Urine dark brown in colour. Reaction neutral. No albumin. Dye present.

c. 4 grm. = 3 grm. per kilo. body weight, given daily in the food, to a Rabbit weighing 1,400 grm. from March 10th to March 28th. No effect was produced, and no albuminuria occurred.

d. 1 c.c. saturated solution injected subcutaneously into Frog (40 grm.).
No immediate or after effects.

15. Helianthin. (free base), occurs in the form of minute, shining violet coloured needles.

a. Jan. 6th 1897. Rabbit. Weight 1,820 grm.
12 p.m. Condition normal. P.204. R.96. T.38.6°C.
12.15. 1.55 grm.= $\frac{5}{6}$ grm. per kilo. body weight injected into stomach in usual manner.
12.30. P.192. R. 98. T. 38°C. No other change except slight dilatation of vessels of ear. Frequent observations made till
5 p.m. but no change observed.
Jan. 7th. 11 a.m. Rabbit perfectly well. About 35 c.c. urine passed during the night. Reaction neutral. Dye present, but no albumin or blood. There was no change during this or succeeding days.

16. Tropaeolin OO. Benzene sulphonate of sodium azo di phenylamine.

$$C_6H_4\begin{cases}SO_3Na \\ N{:}N\text{-}C_6H_4\text{-}NH\text{-}C_6H_5\end{cases}$$

is a yellowish orange powder which dissolves, but not very easily in cold water, forming an orange red solution. It is largely used in wool and

silk dyeing.

a. Roach was killed by a solution of 1 in 2,000, with much suspended matter, in from 12-24 hrs. There was slight external staining.
Solution of 1 in 5,000, with slight suspension did not affect roach in 24 hours.
No staining.

b. Jan. 6th 1897. Rabbit. Weight 2,230 grm. 12.25p.m.Condition normal.P.192.R.84.T.38.1°C. No change till 1 p.m. when temperature was found to be 37.1°C., and ear vessels were somewhat contracted. No further change till 5.p.m. when temperature was found to have risen to 38.4°C.
Jan.7th. 11.15 a.m. Rabbit quite well. There was some pallor and slight yellowness of conjunctivae. About 25 c.c. of pale, brownish green urine passed in which colour could not be detected. Reaction neutral. No albumin. Conjunctivae had lost the yellowish hue by 5 p.m. and animal remained well and lively.

c. 3 grm.= about 2 grm. per kilo. of body weight given daily in food to a Rabbit weighing

1,500 grm. from Feb. 17th to March 8th. Animal occasionally went off its food, but remained well and lively during the whole period. There was doubtful evidence of dye in the urine.

d. 1 c.c. saturated solution injected subcutaneously into a Frog (30 grm.).
No immediate or after effect.

17. Congo Red. Benzidine di naphthionic acid

$$C_6H_4\text{-}N{:}N\text{-}C_{10}H_5\begin{cases}NH_2\\SO_3Na\end{cases}$$
$$|$$
$$C_6H_4\text{-}N{:}N\text{-}C_{10}H_5\begin{cases}NH_2\\SO_3Na\end{cases}$$

is a tetrazo colour derived from the combination of tetrazo diphenyl with naphthionic acid. It is a red brown powder, soluble in water and forming a solution very like diluted blood as it appears turbid. It has a considerable use in cotton dyeing, though it readily changes to a blue colour on the addition of weak acids.

a. Roach and Dace in a solution of 1 in 1000, and in weaker solutions remained unaffected during 24 hours. There was slight external staining.

b. 1 grm. dissolved in distilled water injected subcutaneously with antiseptic precautions into back of a Rat. No effect.

c. 1 c.c. of 1% solution injected subcutaneously into a Frog (33 grm.). No immediate or after effects.

18. Hessian Purple. Diamido stilbene disulphonic

acid < betanaphthylamine
do. do. do.

$$\begin{matrix} CHC_6H_3(SO_3Na).N{:}N.C_{10}H_5(SO_3Na)NH_2 \\ \| \\ CHC_6H_3(SO_3Na).N{:}N.C_{10}H_5(SO_3Na)NH_2 \end{matrix}$$

is a violet coloured powder which has a considerable use in commerce. It is not very soluble in water.

a. Roach was killed by a turbid solution of 1 in 5000 in 7 hours. There was external staining and congestion and clogging of the gills by the suspended matter present in the solution. A clear solution of 1 in 25,000 had no effect on roach within 24 hours. There was slight external staining.

b. 1 c.c. saturated solution injected subcutaneously into a Frog (27 grm.). No immediate or after effects.

19. Primuline. Sodium sulphonate of a primuline base and a toluidine derivative. It is a lemon yellow coloured powder which is soluble to some extent in water forming a yellow solution. It is used in wool, silk and cotton dyeing.

a. Roach in a turbid solution of 1 in 500 were killed in from 12 to 24 hours. Similar weaker solutions produced no effect within 24 hours.

b. 1 c.c. saturated solution injected subcutaneously into a Frog (32 grm.). No immediate or after effects.

20. Rosazurine G. Toluidine / (methyl B naphthylamine delta sulphonic acid
Do. Do. Do.

$$\begin{matrix} C_6H_3(O\ C_2H_5).N{:}N.C_{10}H_5(SO_3Na)NH(CH_3) \\ | \\ C_6H_3(O\ C_2H_5).N{:}N.C_{10}H_5(SO_3Na)NH(CH_3) \end{matrix}$$

is a dark crimson powder used in the dyeing of cotton, and often along with phosphate or stannate of soda, or Turkey red oil. Unlike the solutions of the other colours, the solution of rosazurine G was strongly alkaline.

a. Roach was killed by a solution of 1 in 5000, containing a slight deposit, in 24 hours. There was no staining.

21. Heliotrope. Dianisidine / { methyl naphthylamine delta sulphonic acid.
\ { methyl naphthylamine delta sulphonic acid.

is a violet powder, neutral in reaction, and comparatively soluble in water. It is also used in cotton dyeing.

a. Roach in dark, clear solutions of 1 in 1000 and 1 in 5000 remained unaffected for 24 hrs. There was very slight external staining.

22. Ponceau R.R. Beta naphthol disulphonic acid and diazo metaxylene

$C_6H_3(CH_3)_2.N:N.C_{10}H_4(OH)(SO_3Na)_2$

is a scarlet powder not very soluble in water. It is largely used in the dyeing of wool. The sample used was a laboratory preparation.

a. Gudgeon were killed within 24 hours by clear solutions of 1 in 10,000, and 1 in 100,000, which however had a scum on the surface, and in 24 hours by a similar solution of 1 in 1,000,000.

There was both external and internal staining, the brain and spinal cord being specially affected.

b. ·1 grm. in distilled water injected subcutaneously, with antiseptic precautions, into the back of a Rat. No immediate effects. Rat alive and eating freely 8 hours afterwards, though apparently a little seedy, eyes being half closed and movements sluggish. Urine a bright crimson colour and contained dye. Animal found dead on the following morning.

Post mortem. Muscles, skin, intestines, and all the internal organs except the brain stained a deep red colour. It was rather

difficult to say whether or not the brain was stained, as it did not appear quite of a normal colour. Microscopic examination shewed staining to be diffuse, and the liver to be congested.

23. Azo phenyl Blue.

is a bluish powder, not very soluble in water.

a. Gudgeon in a solution of 1 in 10,000 in which there was a little suspended matter, remained unaffected during 24 hours, and were not stained.

IV. Triphenyl Methane Group.

Triphenylmethane $C\begin{cases}C_6H_5\\C_6H_5\\C_6H_5\\H\end{cases}$ and its analogues are the "parent" substances of this series of important dye stuffs, which may be divided into three sub groups, the Rosanilins, the Rosolic Acids, and the Phthaleïns.

The dyes contain the chromophor ≡ C-NH- or ≡ C-O-, and are obtained as follows.

If amido or hydroxyl groups are introduced into triphenylmethane in certain positions, colourless "leuco" compounds are formed. These, on oxidation yield colour bases, which differ from the "leuco bases" by containing one atom of oxygen. These colour bases, which are usually colourless, unite with acids, with elimination of water, to form coloured salts, which are the real dye stuffs.

A. Rosanilins. All these are basic dyes & derivatives of triphenylmethane and its homologues. They are of various colours and considerable importance being, as a rule, more soluble in water than the azo colours, a fact indeed which applies to the whole group.

1. Malachite Green. The zinc chloride of the colour base, Tetra methyl di amido tri phenyl carbinol.

$$C_6H_5C\begin{matrix}\diagup C_6H_4\text{-}N\ (C\ H_3)_2 \\ \diagdown C_6H_4\text{-}N\ (C\ H_3)_2Cl\end{matrix}$$

is a crystalline green substance with a yellowish green lustre. It is readily soluble in water and is much used in silk and wool dyeing, while it is mordanted on cotton with tannin, and alumina mordants, or tartar emetic.

a. Roach and gudgeon were killed by dark, clear solutions of 1 in 5000 and 1 in 25,000 in 1½ hours; of 1 in 50,000 and 1 in 100,000 in 2 and 4 hours respectively; by a light coloured solution of 1 in 200,000 within 12 hours, while a similar solution of 1 in 1,000,000 had no effect within 24 hours. In every fatal case much mucus was excreted, which became deeply stained, and there was also marked internal staining.

b. ·1 grm. dissolved in distilled water was injected subcutaneously with antiseptic precautions into the back of a Rat. Weakness and dyspnoea rapidly ensued, and death resulted in 35 minutes.

Post mortem. There was brilliant staining of the peritoneum and all the abdominal organs. Muscles, heart and brain were dark in colour, but not stained. Lungs were unstained. Localised staining may possibly be accounted for by diffusion through peritoneal lining with which the needle point was in contact, though there was no perforation.

2. Methyl Green. Chlor methylate of hexa methyl para rosanilin chloride

$$C \begin{cases} C_6H_4\text{-}N\ (CH_3)_2 \\ C_6H_4\text{-}N\ (CH_3).\ CH_3Cl + Zn\ Cl_2 \\ C_6H_4\text{-}N\ (CH_3)_2Cl. \end{cases}$$

occurs as small green crystals, or a light green powder. It is soluble in water and its uses resemble those of Malachite Green, but it requires to be mordanted on wool with sulphur, &c.

a. Roach and gudgeon were killed by a dark, clear solution of 1 in 10,000 within 24 hours. 1 in 100,000 had no effect during that period. In fatal cases, results were similar to those obtained with Malachite Green, but there was no internal staining.

b. Rabbit. Weight 1,340 grm.

2p.m. 2·7 grm.= 2 grm. per kilo. injected into stomach in usual manner.

2.40 Animal shewing signs of weakness and lying on its side.

3 Animal recovering.

3.30 Apparently well and continued so till 5.

10a.m. Following morning Rabbit looked ill. No

food taken and very little urine passed. No other symptoms. Dye not found in the urine.

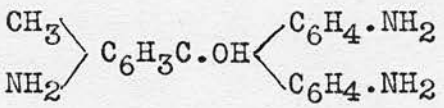
12 p.m. As above.

1 a.m. Animal much livelier. Soon quite well, and continued so.

c. ·1 grm. dissolved in distilled water and injected with antiseptic precautions into the back of a Rat. Marked weakness rapidly ensued, and animal died without other symptoms in 2 hours.

Post mortem. Congestion of liver and kidneys. No staining save locally, i.e. at seat of injection. Urine in bladder contained the dye stuff.

3. Rosanilin. Tri amido tolyl di phenyl carbinol



The hydrochloride is the common dye stuff. It occurs as large octohedra which have a green metallic reflex, and are soluble with difficulty in cold water, giving a fine red colour. It is

employed in wool and silk dyeing.

a. Gudgeon was killed by a clear solution of 1 in 10,000 within 24 hours. Much mucus was excreted which was deeply stained, but there was no other staining. A similar solution of 1 in 100,000 produced no effect within 24 hours.

4. Rosanilin Acetate.- $C_{20}H_{19}N_3C_2H_4O_2$ is the most soluble rosanilin salt.

a. Gudgeon was killed by a clear solution of 1 in 10,000 within 24 hours. A similar solution of 1 in 100,000 produced no effect in that period. There was slight external staining in the first case.

5. Rosanilin or Gentian Blue. Tri phenyl rosanilin chloride.

$$C_{20}H_{16}(C_6H_5)_3N_3Cl$$

is a dark violet powder very slightly soluble in water, and as a result the experiments with it were unsatisfactory, though they tended to prove

that suspended matter has probably little effect.

a. Roach in half solutions, half suspensions of 1 in 5000, and 1 in 10,000 remained unaffected during 24 hours. There was no staining.

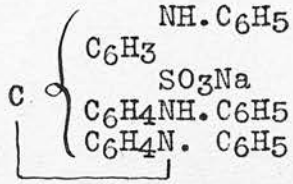
6. China Blue. Sodium salt of di & tri sulphonic acids of tri phenyl rosanilin and tri phenyl para rosanilin

(see Alkali Blue.)

is a dark, blue powder soluble with difficulty in water, but rendered more soluble by previous trituration.

a. Roach in very dark but clear solutions of 1 in 1000 and 1 in 5000 remained unaffected during 24 hours, but in the first case the fish died on the following day with no symptoms beyond those of an increasing weakness. It was transferred to pure water at the end of 24 hours. There was external, but no internal staining.

7. Alkali Blue. Sodium salt of the mono sulphonates of mono, di, & tri phenyl rosanilin & para rosanilin.



is a blue, amorphous powder, chiefly used in wool dyeing.

a. Gudgeon in a solution of 1 in 5000 (limit of solubility) remained unaffected for 24 hours. No staining.

8. Hofmann's Violet. Tri ethyl rosanilin.

$$C_{20}H_{18}(C_2H_5)_3N_3O$$

is in the form of green needles, and is sparingly soluble in water. It is not now much used as a dye.

a. Gudgeon was killed by a dark, clear solution of 1 in 10,000 in 6 hours, and by a similar solution of 1 in 100,000 in 24 hours. There was a slight excretion of mucus, and slight external staining. No internal staining.

B. Rosolic Acids. These may be regarded as rosanilins in which the nitrogen is replaced by a group containing oxygen.

1. Rosolic Acid. $C_{20}H_{16}O_3$
occurs as lustrous green crystals forming with difficulty a red solution.

a. Roach was killed by a clear solution of 1 in 10,000 in 3 hours. A similar solution of 1 in 100,000 was not fatal in 24 hours but caused considerable weakness.

b. ·1 grm. dissolved in distilled water injected subcutaneously with antiseptic precautions into back of a Rat. No effect.

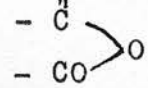
2. Aurin or Yellow Corallin.

{Sodium salt of a mixture of {Aurin= $C_{19}H_{14}O_3$ Methylaurine Pseudo rosolic acid

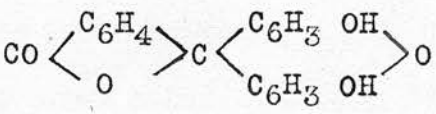
occurs as reddish brown masses, and is chiefly used in wool, silk, and calico printing.

a. Roach was killed by a clear, deep red solution of 1 in 5000 in 2½ hours, by a similar solution of 1 in 10,000 within 24 hours, fish

shewing signs of great weakness in 6 hours, while a pale pink solution 1 in 100,000 produced no effect in 24 hours. In first case there was slight external staining.

C. Phthaleïns. These have a different chromophor from either of the other Triphenyl methane groups, namely  They constitute a very large group, but only a few of them are of importance as dyes.

1. Fluoresceïn. Anhydride of tetra oxy di phenyl phthalide



occurs as dark yellow crystals, but, not being fast, is not much used commercially.

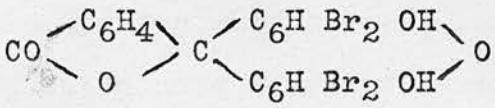
a. Gudgeon in clear, fluorescent solutions of 1 in 5000, and 1 in 10,000 remained unaffected during 24 hours. No staining.

Note. The dye which follows was obtained from a chemical laboratory. At first it

was thought to be the same as another sample, but they differed markedly in colour, the former being yellow, the latter salmon coloured. They had different effects on fish.

Probably the second was impure and the results obtained with it as not included here.

2. Eosin (Yellow shade). Tetra Brom Fluorescein.



was a yellow powder, acid in reaction, and fairly soluble after trituration.

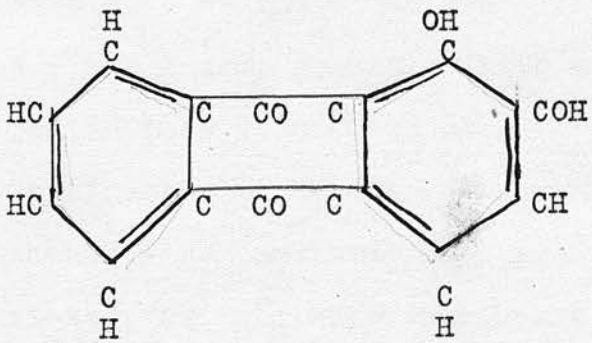
a. Roach and gudgeon in clear, fluorescent solutions of 1 in 5000, and 1 in 10,000 remained unaffected during 24 hours. No staining.

b. .1 grm. suspended in distilled water injected as usual into Rat. No staining.

V. Oxyquinones and Ketoneimides.

A. Oxyquinones. The quinones (di ketones) which have a double CO group, belong to the most powerful class of chromogens. They yield actual dye stuffs by the introduction of auxochromic groups. The oxyquinones possess a specially marked dye stuff character, as the quinone group belongs to the acid forming chromophors, and the hydroxyl group introduced develops powerful acid properties. In dyeing they are only employed along with mordants.

1. Alizarin. Di oxy anthra quinone = $C_{14}H_8O_4$



is one of the few natural dye stuffs which have

been prepared synthetically. It occurs as a glucoside chiefly in madder, the root of rubia tinctorium. It is an anthracene colouring matter, anthracene being oxidised to anthraquinon and alizarin obtained from the sulphonic acids of the latter. The sample used was a brownish yellow powder dissolving very easily to a fine red solution, neutral in reaction. Its chief use is in Turkey red dyeing.

a. Roach was killed by a dark solution, of 1 in 10,000, having a cloud at the bottom, within 24 hours. There was no staining. A perfectly clear solution of 1 in 100,000 did not affect a gudgeon in 24 hours.

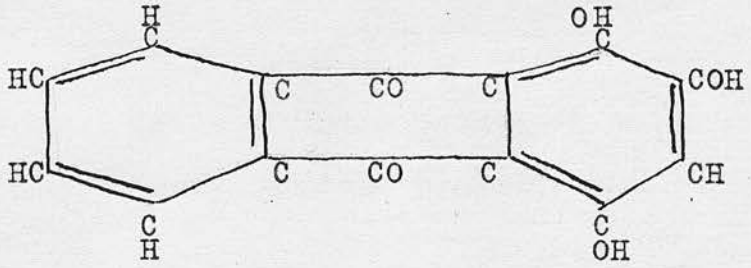
b. Rabbit. Weight 1,320 grm.

12.30p.m. 4 grm.= 3 grm. per kilo. body weight injected into stomach as usual.

1.30 No effect till this time when animal looked ill, remaining with eyes half closed, and its movements being sluggish Continued in this condition till night, but took food fairly readily. Next day seemed perfectly well and continued so. Urine contained the dye.

c. .1 grm. in distilled water injected as usual into a Rat. No effect.

2. Purpurin. Tri oxy anthra quinone. $C_{14}H_5O_2(OH)_3$



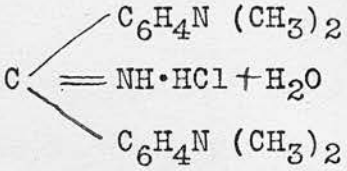
As shewn in the constitutional formula the 3 hydroxyl groups are in one benzene ring. It is a reddish yellow powder, not very soluble in water, and faintly acid in reaction.

a. Gudgeon in a 1 in 5,000 solution (limit of solubility) remained unaffected during 24 hrs. There was no staining.

B. Ketoneimides. The dye stuffs of this group are derived from the simple ketones, as the quinoneimides are derived from the quinones. In them, however, the oxygen of the CO group is replaced by a nitrogenous group. Otherwise they would not be dyes, as the CO group, occurring in an open carbon chain, cannot act as a chromophor, unless its oxygen be replaced either as above or by a

sulphur group. They are chiefly used in cotton dyeing and are used along with mordants, such as alum and tartar emetic.

1. Auramine. Imide of tetra methyl di amido di phenyl methane hydrochloride.



is a fine yellow powder, neutral in reaction, and fairly soluble in water.

a. Roach and gudgeon in a clear solution of 1 in 5000 were killed in 2½ hours; of 1 in 10,000 in 24 hours.

There was very brilliant and complete external staining, and there was also internal staining, especially of brain and spinal cord.

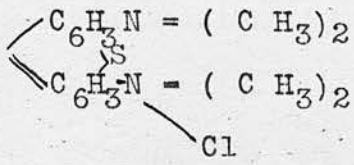
The fish exhibited symptoms of convulsive spasm to a marked extent.

VI. Quinoneimide Group. The dye stuffs of this group are complex derivatives of simple quinoneimide

which theoretically is obtained by replacing the two oxygens of quinone by imido groups, as yet it has not been isolated, but simple compounds (e.g. quinone chlorimide) are known.

A. Indophenols. The only member of this group employed was one containing one molecule of sulphur, viz.

1. Methylene Blue.



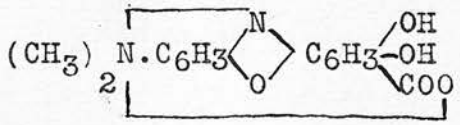
is a bluish violet powder, soluble in cold water, and the sample used had apparently a slightly acid reaction.

a. Roach was killed by a very dark blue solution of 1 in 10,000 in from 4 to 6 hours. There was slight external and internal staining. Solutions of 1 in 50,000 and 1 in 100,000 had no effect on either roach or gudgeon during 24 hours. The nasal pits were specially stained.

b. ·1 grm. in distilled water injected as usual into a Rat. No effect. Dye present in the urine.

B. Oxyindamines. In these an oxygen atom takes the place of the sulphur atom of the thioindamines. Some are obtained from gallic acid, i.e.

1. Gallocyanin.

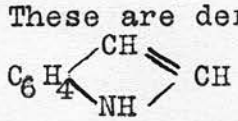


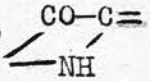
The sample obtained was in solution, the strength of which could not be ascertained. As a result the experiments only go to prove that it cannot be very toxic to fish. The sample solution was diluted. This dye is chiefly used in calico printing.

a. Roach in nearly colourless solutions of 1 in 1000, and 1 in 5000 remained unaffected during 24 hours. No staining.

VII. Indigo and Quinoline Groups.

A. Indigo colours. These are derived from Indol -



and their tinctorial properties are due to their possessing the chromophorous group  They are used for both wool and cotton, and are employ-

ed with reducing agents in the process known as vat dyeing.

1. Indigo carmine. Indigotine di sulphonic acid.

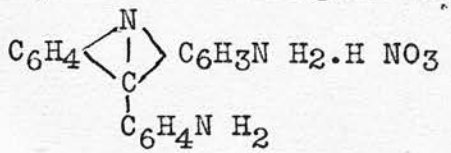
$$C_{16}H_8N_2O_2\ (SO_3H)_2$$

is an amorphous powder, soluble in water, and is chiefly employed in an alum bath for wool dyeing.

a. Roach in a clear, deeply coloured solution of 1 in 1000 was not killed within 24 hours, but was considerably weakened. There was slight external staining. A roach in a similar solution of 1 in 5000 remained quite unaffected during 24 hours.

B. Quinoline Colours. Quinoline C_9H_7N bears the same relation to naphthalene as pyridine to benzene, i.e., one CH group is replaced by a N. atom. Acridine, $C_{13}H_9N$ an allied substance, bears the same relation to anthracene as quinoline to naphthalene.

1. Phosphine, though here put under the Quinolines is more correctly an acridine dye stuff, being a mixture of the nitrates of chrysanilin = di amido phenyl acridine, and of homologous bases.

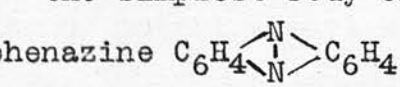


is a yellow powder, easily soluble in water and finding its chief application in silk dyeing and woollen printing.

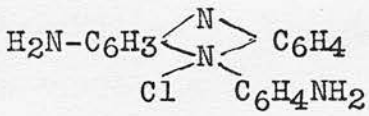
a. Roach were killed by dark, yellow solutions of 1 in 5,000 and 1 in 10,000, which shewed a very slight suspension, in 20 minutes, by clear solutions of 1 in 50,000 and 1 in 100,000 in 4 hours, while a solution of 1 in 1,000,000 was not fatal. In the first two cases there was external, but no internal staining. In the third and fourth cases there was both external and internal staining, the brain and spinal cord being specially affected.

b. .1 grm. dissolved in distilled water injected as usual into a Rat. No immediate or after effects.

VIII. Azine and Induline Groups.

A. Azine Colours. The chromophor of this series is the group  the simplest body of the azine group being phenazine 
The simplest azines are not dyestuffs, but the introduction of amido or hydroxyl groups increases their tinctorial power.

1. Saffranin. Para amido phenyl para amido phenazonium chloride.

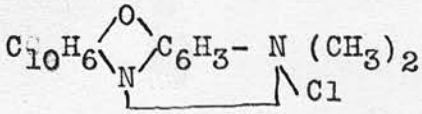


belongs to a special group as it contains four nitrogen atoms. It is a brownish red powder, fairly soluble in water and is used in wool and silk dyeing, while it is also mordanted on cotton, usually with tannin and tartar emetic.

a. Roach were killed by a dark red, clear solution of 1 in 10,000 within 24 hours. Mucus was excreted which was stained, but there was no other staining. A solution of 1 in 100,000 had no effect during that time.

b. .1 grm. dissolved in distilled water was injected as usual into a Rat. No immediate or after effects.

2. New Blue. Chloride of di methyl phenyl ammonium B naphthoxazine.

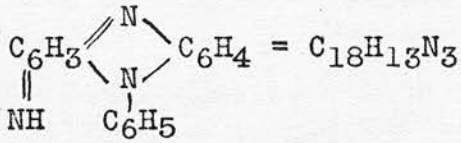


is a dark violet powder, which in dust form, powerfully affects the mucous membranes. It is soluble

in water, and is chiefly used along with the common mordants in the dyeing of cotton.

a. Roach was killed by clear, dark purple solutions of 1 in 5,000 and 1 in 10,000 in one hour, by a solution of 1 in 100,000 in 6 hours. In all these cases a large quantity of mucus, which was deeply stained, was excreted. There was no internal staining. A solution of 1 in 500,000 had no effect and produced no staining.

B. Induline Colours. They are produced by the action of Azo, nitro, and nitroso compounds on aromatic amines, and bear a relation to the azine group, containing from three to five atoms of nitrogen in the molecule. The simplest has the constitution



$C_6H_3 \begin{matrix} \diagup\!\!\diagup N \diagdown \\ \diagdown N \diagup \end{matrix} C_6H_4 = C_{18}H_{13}N_3$

NH C_6H_5

They form red, violet, or blue dyes.

1. Nigrosine is produced by the action of nitro compounds on anilin hydrochloride in the presence of ferrous chloride. It is an almost black powder, soluble in water and is used in silk and wool dyeing.

a. Gudgeon was killed by a clear violet blue solu-

tion of 1 in 10,000 within 24 hours. There was very slight external, and no internal staining. A solution of 1 in 100,000 produced no effect during 24 hours.

b. .1 grm. dissolved in distilled water was injected as usual into a Rat. No immediate or after effects.

The only mineral colours at present used to any extent are the following:-

1. Chrome Yellow or chromate of lead - $Pb\ Cr\ O_4$
2. Chrome Orange - $Pb_2Cr\ O_5$ - normal or basic lead chromates.
3. Chrome Green - Chromium hydroxide (rarely employed)
4. Manganese Brown or Bronze - Hydrated peroxide of Manganese.
5. Iron Buff and Nankin Yellow - Both hydrated ferric oxide.

The toxic properties of these substances are well known, and no experiments were made with them.

One vegetable substance was tested on Fish, namely Black Catechu or Cutch.

A Roach was killed by a solution of 1 in 10,000, in which there was a slight precipitate, within 24 hours. There was no staining or congestion of the gills.

A solution of 1 in 100,000 was not fatal during that period.

The Experiments with Mordants will be found in the Table in Appendix II, and do not call for detailed description. Some as stated were unsatisfactory. The others gave definite results, and apart from their interest as mordants, appear useful as compared with the toxicity of the dyes, most of them being well known substances.

As a Control Experiment Corrosive Sublimate was test-
on Fish.

A Roach was killed by a solution of 1 in 10,000 in 65 minutes, by a solution of 1 in 100,000 in 2½ hrs. In both cases the chief symptom was urgent dyspnoea, and nothing special was found on post mortem examination.

The Experiments with Mill Effluents and River Waters will also be found in the tables. The contained substances were, for the most part, very complex, and some notes on their effects will be found in the "Remarks" which follow.

REMARKS.

In attempting to draw some conclusions from the results of the experiments and a comparison between these results and those of other observers, it appears best to consider each group in the first place separately, and in much the same order as previously adopted.

I. The Nitro Group (No_2) is undoubtedly poisonous but it is certainly doubtful if, in all cases, the toxicity is due to the number of NO_2's contained in the molecule. In similarly constituted bodies this may be the case as long as the compounds remain fairly soluble. Di nitro benzols are somewhat more poisonous than nitro benzol (Rohl, Dissertation, Rostock 1890) but in these experiments and also in Weyl's, Martius Yellow (2 NO_2's) is more toxic than Picric acid (3 NO_2's). A strict comparison cannot however be drawn here, as in the one case the naphthol group is present, in the other, the phenol. The introduction of a nitro group into a body without one, probably always increases the toxic effect. This is shewn in the experiments under the azo group with Orange G and Orange III, both in the case of fish and rabbits.

Other negative groups i.e. Carboxyl (COOH) and Sulpho groups (sulphonic especially) diminish the toxicity. The latter fact is shewn by a comparison of the results obtained with Martius Yellow and Naphthol Yellow S, and is borne out by Weyl's experiments with these two dyes and also with Brilliant Yellow. The same thing is seen in other compounds. Thus sodium oxy methyl sulphonate is less toxic than formaldehyde.

Aurantia, about the toxicity of which there was considerable doubt, certainly seems poisonous. It is so in a very marked degree to fish, especially affecting the nervous system. In the one experiment performed on a rabbit, 3 grm. per kilo. body weight caused well marked catarrhal nephritis, which resulted fatally in 36 hours. There was also an irritant condition of the mucous membrane of the large intestine produced. This, taken along with the statements regarding its effects upon the skin, makes one look upon it as a fairly irritant poison.

With regard to Poirrier's Orange, also very fatal to fish, it is interesting to note that all nitranilines are decidedly poisonous, and paranitranilin is much more poisonous than meta- or ortho- (p-nitranilin kills animals in .04 grm. per kilo., intravenously.). The same influence of para- meta- and ortho- positions is exhibited, but not to a similar extent in the di-hydroxy benzols.

The Nitro dyes must for the most part be regarded as very fatal to fish and are probably very injurious when passed into streams, though of course, as in all cases it is very difficult to say what effect the various substances with which they become mixed, have on their toxicity. In the lists of injurious colours published in the legal enactments of various countries, they are usually included. Previous to Weyl's Experiments however, the Sonnenberg Chamber of Commerce and Trade permitted the use of Martius Yellow in the painting of toys, regarding it as harmless. I have not been able to determine whether they have seen fit to alter this erroneous view.

II. The Nitroso Group The Nitroso dye here tested is unfortunately hardly typical of the class, the sulphonic group probably exercising most influence, but it is apparent that in a complex molecule the group NOH did not confer toxicity. From the researches of Pomfret (Transactions of the Royal Society) and of Leech (Croonian Lectures, 1893) it appears that the iso nitroso group itself is not very toxic. At least it is not so in its fatty compounds and therefore probably in its aromatic compounds also. According to Gibbs and Reichert however, Nitroso ethylene, a fatty nitroso compound, is poisonous, and Loew states that Nitroso B. Naphthol is very toxic

("Ein naturliches System der Giftwirkung"). Weyl has also shewn di nitroso resorcinol to be a poisonous substance.

III. Azo Group. Many of these are so complicated that it is very difficult to form any definite conclusions regarding them. The "parent" substances, azo benzene and azo toluene are undoubtedly poisonous, and the latter seems to be slightly more so than the former. They are both intestinal and kidney irritants, but it is evident that a very considerable amount would have to be taken in order to produce a fatal result in human beings. In this case the introduction of a methyl (CH_3) group apparently increases toxicity.

On the whole Weyl's experiments agree with mine on mammals, which show that most of the azo dyes are non toxic even in large doses.

Weyl, however, concluded that Tropaeolin 000 (Orange II) was poisonous, while it produced no effect in my research, which in this respect agreed with what was found by Cazeneuve and Lépine.

Ponceau R.R. is certainly a toxic substance, but it belongs to the diazo group which is more toxic than the Azo.

In the case of rabbits the introduction of toluene, xylene, cumene, and naphthalene groups apparently rendered the dye stuffs more hurtful, as was the case when a nitro group was included.

Fast Red B., containing the naphthalene group, seems to have about the same degree of toxicity as Cumidine Red. An additional Azo Benzene apparently lessens the poisonous properties as shewn in the case of Brilliant Croceïn.

The experiments on Frogs proved unsatisfactory, but it was not easy to inject a larger quantity, and one wished to give the same amount on each occasion.

On turning to the experiments on Fish, one must state that, for the most part, the results obtained were somewhat different from those on mammals.

Anilin Yellow (Amido azo benzene) we find to be non toxic even in very strong solutions, while Chrysoidine (Di amido azo benzene) and Bismark Brown

(Tri amido azo benzene) are exceedingly poisonous, and it is curious to find that chrysoidine is the most toxic of the three. It will be noticed that the most powerful action accords with the most intense staining, and there seems to be a curious affinity, possessed by the nervous system of Fish, for some of these dyes. Chrysoidine was more soluble than Bismark Brown, but not so soluble as Anilin Yellow. Of course there may have been some undetected impurity in it, but I am inclined to think its toxicity was due to its rapid absorption and action on the tissues, especially the brain and spinal cord.

Though omitted in the tables, it was found that 1 in 1,000,000 proved fatal to roach in 48 hours with the usual phenomena of staining, and of all the dyes tested Chrysoidine appears the most deadly to fish.

From the experiments with Brilliant Orange and Tropaeolin 000 No.2, one perhaps is justified in concluding that the sulphonic group diminishes toxicity to a less extent when combined with naphthol than with the benzene ring.

While the results obtained from the introduction of a nitro group are comparable with those got from the experiment on a rabbit, the effect of intro-

ducing toluene, xylene, and cumene groups is to lessen the toxicity. Fast Red B.(naphthalene) seems to be more poisonous to fish than Cumidine Red.

Here as in the case of mammals an additional azo benzene appears to lessen toxicity as seen in the experiments with Brilliant Crocein. The other members of the group are so complex that it is impossible in the short space at my disposal, to draw any definite comparisons between their chemical constitution and toxic action. It would seem however as if increase in the size of the molecule lessened toxicity, and one must draw attention to the further evidence as to the poisonous properties of Ponceau R.R

With the exception then of two or three members of the group, the azo dyes must be considered poisonous to fish, though probably they would have little influence in streams owing to their great dilution. An analogy may perhaps be drawn between their action and the results obtained by hypodermic injection, and this probably applies to all the experiments with dyes on fishes. In hypodermic medication toxic effects are more easily produced, as will be seen by reference to Weyl's tables. In fish, which are very susceptible to the dyes, the substances no doubt pass into the blood stream rapidly

and it may be continuously, and thus the marked difference between the results obtained from experiments on them, and from experiments on mammals, in which the dye is introduced by the stomach, may be explained. Of course it may not be correct to compare fish with warm blooded animals, but there does not seem to be any powerful reason against it, as frogs are constantly used in pharmacological and toxicological experiments, and very correct deductions can be drawn from them.

One must direct attention to the frequent onset of fungus growth which is very interesting when compared in the light of the recent disclosures regarding pollution of the river Spey by refuse from distilleries.

It is interesting to note that the Sonnenberg Chamber permit the use of all Ponceaus, and that the French law regard diazo colours, such as Xylidene Red and the Tropaeolins, as poisonous, along with those containing the Nitro group.

IV. Triphenylmethane Group. Of the Rosanilins, Malachite Green and Methyl Green are found to be very fatal, both to rats and fish. They were carefully examined for arsenic, but none was found, and their toxicity may possibly be due to the Zinc Chlo-

ride they contain, though, as will be apparent, malachite green is one of those dyes which produce internal staining.

The other rosanilins do not seem very toxic to fish with the exception of Hofmann's Violet, in which it is possible that the introduction of three ethyl groups increases the toxicity. One was indeed surprised to find in what deeply coloured solutions fish could live, apparently with very little discomfort after they became used to their altered surroundings. Very often the solution was so dark that the fish was not visible, as in the case of China Blue, 1 in 5,000, and yet no harm resulted. The same was found with dyes of other groups. It will be noticed that as a rule mucus was excreted which points to an irritant action on the skin, its excretion being either pathological, or for the purpose of protection.

Malachite Green has hitherto generally been regarded as harmless when pure and both it and Methyl Green and Rosanilin are permitted for the painting of toys by the Sonnenberg Chamber. The sample of Malachite Green which I used was a laboratory preparation, and, as already stated, free from Arsenic.

The Rosolic Acids are certainly fatal to fish

and though these dyes are stated to be non poisonous (vide Introduction) it is significant that their use for foof stuffs, etc. is forbidden both by Austrian and Swiss legislation.

The Phthaleïns are probably non toxic. Eosin is regarded as harmless by the Sonnenberg Chamber.

The dyes belonging to the other groups are very complicated bodies, and I had not an opportunity of testing many of them on mammals.

Auramine and Phosphine are both very fatal to fish and so is New Blue, though the last named did not cause internal staining. All these substances would require prolonged and careful investigation, and I had not the opportunity of making as many experiments with each dye as I should have liked.

On the whole it is probable that most of the dye stuffs are passed into streams in such a diluted condition, and there become so much more diluted, that they do not kill the fish. This is borne out by what is found in the river Leven, in Dumbartonshire, which is polluted by effluents from Turkey Red works. It is said that, in this case, the dye stuffs have no effect on the fish which are injured by caustic and other substances employed in the process. Quite apart from any fatal result however the fish may suffer in health from fungus growth, while deposits are

likely to occur, and as it would seem that simple methods can obviate these undesirable effects, their use should be enforced.

As is shewn in the experiments with mill effluents and stream waters filtration or simple precipitation apparently renders the effluents innocuous. In the case of the Tweed, trout are frequently caught at a point just below where the mill discharges take place, and no objection can be taken to the condition of the river water, although as will be seen by reference to the tables, the dyes and substances mixed with them are very fatal.

Indigo effluents, even when foul smelling, did not kill fish and must be regarded as non toxic; but most of the Yorkshire rivers in the factory districts are in a disgraceful state. Dr Maclean Wilson, Medical Inspector to the West Riding Rivers Board, to whom I am indebted for the samples he sent me, has however devoted attention to the existing state of affairs, and improvement may be looked for. I must also express my thanks to Oscar Schofield Esq. of Littleboro' Lancashire, who sent me samples containing vegetable dyes and mordants. Such a sample was rapidly fatal, but after filtration its toxic properties entirely disappeared. He employs Kremmo-

lite, a form of iron ore for the purpose and from my results it would seem to act admirably.

It is probable therefore that careful filtration or precipitation is all that is required, with a sufficient staff of inspectors to see that, whatever process is adopted, it is efficiently carried out. Till this is done, not only in connection with dyes and mordants, but also with other forms of stream pollution, the great dream of Frank Buckland's life is not likely to be realised.

In conclusion I have to express my obligation to Professor Bradbury for kindly giving me permission to pursue in his laboratory, this research, which incomplete and imperfect, though it is, may go a little way in adding to our knowledge of the toxicity of dye stuffs, and excite some interest in the important and pressing question of river pollution.

A P P E N D I X. I.

Laws regulating the Use of Poisonous Colours.

A P P E N D I X I.

Laws regulating the Use of Poisonous Colours.

Colours.

This Appendix is not intended to give a full list of the various legal enactments in force regarding the use of colouring matters. It is merely a brief resumé of the principal acts in force in various countries, to shew the importance of the subject and the manner in which the various governments have dealt with it. Long lists, such as are set forth in Weyl's work are avoided, as it is intended to convey merely a general idea of what has been done, and not to enter into minute particulars.

Great Britain and Ireland.

This subject is dealt with in section 3 of the Sale of Food and Drugs Act 1875, which provides that "no person shall mix, colour, stain, or powder (or order or permit any other person to mix, colour, stain or powder) any article of food with any ingredient or material so as to render the article injurious to

health, with intent that the same may be sold in that state, and no person shall sell any article so mixed, coloured, stained or powdered, under a penalty, not exceeding £50 for a first offence, and on a subsequent conviction, of imprisonment with hard labour for a period not exceeding six months."

In the Amendment Act of 1879, the method of proceedure in such a case is set forth.

The use of Anilin colours, unless strongly arsenical, is not forbidden.

Austria-Hungary.

The Austrian laws are the most stringent, and they set forth very fully the regulations in force, giving lists of harmless and harmful colours with regard to the painting of toys, the colouring of confectionery and food, household articles, etc. The older laws bear extensive reference to mineral and vegetable dyes; of the more recent the following may be noted.

1. The Royal Austrian Ministerial publication of May 1st 1886 restricts the choice of those colours which were previously permitted for the colouring of food. This agrees fairly well with the German enactment of Dec. 5th 1887.

2. The Austrian minister for the Home Department forbids (1887) the use of Rosolic Acidsto colour eatables.
3. In a special Government Book 1887, 35, a publication of the same minister forbids the use of colours, obtained from Anilin or (organs of) animals, to colour egg shells which are to be used.
4. In later Acts Anilin Red is forbidden as a dye for children's whistles, and in Hungary Dinitrocresol and Corallin are not permitted as colouring matters for food.

Belgium.

The use of poisonous colours to colour food,etc. is forbidden, as is the sale of foods coloured with poisonous dyes, a list of which is given.

France.

As the result of Wurtz's work (1881) the law on the use of colour to colour food, etc. was remitted. A list of poisonous and permitted colours is added. It appears that in later years a milder practice has existed.

Germany.

The German Law is fairly complete but in need of revision. It deals with the use of colours for food and drink, vessels, wrappers, and covers, toys, paints, etc. It also refers to book printing and lithographing, the manufacture of sacred wafers etc, and contains clauses dealing with the penalties enforced, and lists of poisonous and non-poisonous colours. In addition it sets forth methods for the detection of arsenic and tin in artificially coloured articles of food and drink, and refers specially to the use of arsenical dyes.

Italy.

Here we find a ministerial resolution which depends on the public health law of 1888, and which gives three lists of poisonous colours, which are not to be used; the first for foods, etc., the second for toys, and the third for furnishing materials (curtains etc).

Switzerland.

For Canton Berne, according to a decree of 15th Nov.

1892, Picric Acid, Dinitrocresol, Martius Yellow, Aurantia, Orange II, Metanilin Yellow and Saffranin are forbidden to be used for colouring food etc. The Swiss analytical chemist's society adds Methylene blue and Ethylene blue.

The above brief extract will give some idea of the amount of attention which has been directed to this important subject. Very little has been done with regard to the fouling of streams by dye effluents. In this country the Rivers Pollution Prevention Act of 1876 deals to some extent with the question, and it is satisfactory to observe that lately, attention has been directed to the matter, and it is probable that more complete and stringent regulations will ere long be put in force.

A P P E N D I X II.

Tabulated Lists of Principal Experiments.

NITROSO AND NITRO COLOURS.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution.	Lethal Period.	Remarks
1. Naphthol Green B. (Sodium Ferrous nitroso naphthol sulphonate.)	Neutral	Roach.	1 in 1000 } clear but deep coloured green solutions	No effect.	No staining. Fish remained lively.
			1 in 5000 }	No effect.	
1. Picric Acid.	Acid	Roach	1 in 10,000 } clear yellow solutions	Within 24hrs.	Slight external staining. No internal staining.
			1 in 100,000 }	No effect.	No staining.
2. Martius Yellow (Di nitro - A - naphthol)	Neutral	Gudgeon	1 in 10,000 some suspension	One hour.	No staining produced by any of the solutions.
			1 in 100,000 } clear solutions	Within 24 hrs.	
			1 " 1,000,000 }	No effect.	
3. Naphthol Yellow. S. (Sodium di nitro - A - naphthol sulphonate	Neutral	Gudgeon	1 in 5,000 clear solutions	No effect even though fish showed fungus growth.	Fungoid part stained yellow. No other part stained.
4. Aurantia. (Sodium salt of hexa nitro di phenylamine.)	Neutral	Roach	1 in 5,000 slight suspension.	1½ hours.	Complete external staining. Also internal staining, especially of brain and spinal cord.
			1 in 10,000 } clear yellow	2 "	
			1 in 100,000 } solutions	6 "	
			1 in 500,000 solution shows scarcely any colour.	24 "	Same with all the solutions Well marked spasm and twitchings.

Table of Experiments on Fish. (cont)

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks
5. Poirrier's Orange. (Meta nitranilin B naphthol disulphonic acid.)	Neutral	Roach.	1 in 5,000 dark clear yellow solution 1 in 10,000 clear yellow solution. 1 " 100,000 slightly coloured solution.	4 hrs. ? fatal. No effect.	Slight external staining, specially of fins and tails. No internal staining.

AZO, OXYAZO AND TETRAZO COLOURS.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish.	Strength of Solution.	Lethal Period.	Remarks.
1. Anilin Yellow. (Amido azo benzene.)	Neutral	Roach & Gudgeon	1 in 200 clear deep red solutions. 1 in 500 clear deep red solutions. 1 in 1,000 clear deep red solutions.	No effect do. do.	External staining i.e. of scales and gill covers.
2. Chrysoidine. (Di amido azo benzene.)	Neutral	Roach & Gudgeon	1 in 1,000 clear yellow solutions. 1 in 10,000 clear yellow solutions. 1 in 100,000 clear yellow solutions. 1 in 200,000 clear yellow solutions.	A few minutes. do. do. 4 to 6 hours. 10 - 24 "	All fish stained both externally and internally. Muscles, brain, spinal cord &c. a bright yellow.
3. Bismark Brown (Tri amido azo benzene.)	Neutral	Roach & Gudgeon	1 in 10,000 fairly clear yellow solutions 1 " 100,000 fairly clear yellow solutions 1 " 200,000 fairly clear yellow solutions 1 " 1000,000 fairly clear yellow solutions	30 minutes. 45-60 " No effect No effect.	Well marked yellow external staining. Slight staining of muscles, brain &c. in fatal cases.
4. Brilliant Orange. (Benzene azo B Naphthol sulphonate of sodium.	Neutral	Roach & Dace.	1 in 5,000 fine suspension 1 " 10,000 fairly clear solution 1 " 20,000 clear solutions. 1 " 30,000 clear solutions.	5 hrs. 6 " Within 24 hrs. No effect.	Deep staining of fins and neutral surface in all cases. No internal staining in fatal cases. Congestion of gills present however.
5. Tropaeolin 000. No.2 (Benzene sulphonate of sodium azo B naphthol.)	Neutral	Roach	1 in 5,000 fine suspension 1 " 10,000 fairly clear solution.	24 hrs. No effect.	Fish stained externally. No internal staining. Not much staining of scales and fins.

Table of Experiments on Fish (cont.).

Substance	Reaction	Species of Fish	Strength of Solution.	Lethal Period.	Remarks.
6. Orange G. (Benzene azo B naphthol di sulphonate of sodium)	Neutral	Roach & Gudgeon	1 in 1,000 clear yellow solutions 1 " 5,000 clear yellow solutions	3 hrs. No effect	Ventral surface and fins stained. No internal staining. Fish died next day. Fungus growth on snout.
7. Orange III. (Nitro benzene azo B naphthol di sulphonate of sodium	Neutral	Roach	1 in 5,000 fine suspension	5 hrs.	Immediate signs of respiratory difficulty. Gills congested and clogged with mucus. No staining.
8. Orange G.T. (Toluene azo B naphthol sulphonate of sodium.)	Neutral	Roach & Dace.	1 in 5,000 turbid solution. 1 " 25,000 clear solution	12-24 hrs. No effect	Slight external staining. Gills congested. No internal staining.
9. Xylidene Red. (Xylene azo B naphthol di sulphonate of sodium)	Neutral	Roach	1 in 1,000 clear solution with slight fluffy deposit. 1 in 5,000 clear red solution.	24 hrs. No effect	Brilliant external staining. No internal staining. Slight external staining.
10. Cumidene Red. (Cumene azo B naphthol di sulphonate of sodium.)	Neutral	Roach	1 in 5,000 clear dark red solutions 1 in 10,000 clear dark red solutions	No effect No effect	Fish died 4 days afterwards with fungus growth & loss of stained scales. Slight external staining.
11. Fast Red B. (A naphthalene azo B naphthol disulphonate of sodium)	Neutral	Roach	1 in 1,000 Slight suspension 1 in 5,000 dark, clear purple solution.	12-24 hrs No effect	Deep external staining. No internal staining. Special staining of certain scales. Fish died in 7 days with loss of these scales & fungus growth.

Table of Experiments on Fish (cont).

Substance	Reaction	Species of Fish	Strength of Solution.	Lethal Period.	Remarks.
12. Biebrich Scarlet (Benzene sulphonate of sodium azo benzene sulphonate of sodium.)	Neutral	Roach	1 in 5,000 slight suspension	No effect.	Fish lost its tail. Stump deeply stained.
			1 in 10,000 very turbid solution.	No effect	Tail alone, deeply stained.
13. Brilliant Crocein (Benzene azo benzeneazo B naphthol di sulphonate of sodium)	Neutral	Roach	1 in 500 } both clear 1 in 1,000 } solutions.	12-24 hrs No effect	Bright red external staining. Slight yellowish internal staining. Fish with roe, however, killed in 24 hours.
14. Helianthin (Benzene sulphonate of sodium azo di methyl aniline	Neutral	Roach	1 in 5,000 } both turbid 1 in 10,000 } solutions	24 hrs. No effect	Slight external staining. No internal staining.
15. Tropaeolin 00. (Benzene sulphonate of sodium azo di phenylamine)	Neutral	Roach	1 in 2,000 solution with much suspended matter	12-24 hrs	Slight external staining
			1 in 5,000 slight suspension	No effect	No staining.
16 Congo Red. (Benzidine di naphthionic acid)	Neutral	Roach & Dace	1 in 1,000 } blood red solutions, turbid even after filtration.	No effect	Slight external staining in all three cases.
			1 in 5,000	No effect	
			1 in 25,000	No effect.	

Table of Experiments on Fish (cont.).

Substance	Reaction	Species of Fish	Strength of Solution.	Lethal Period	Remarks.
17. Hessian Purple. (Di amido stilbene disulphonic acid di beta naphthylamine.	Neutral	Roach	1 in 5,000 turbid solution 1 in 25,000 clear solution.	7 hrs. No effect.	Slight yellowish and "mother of pearl" staining of scales. Gills clogged and congested in fatal cases.
18. Primuline. (Sodium solphonate of primuline base=sulphur & para toluidine.) ∴ A Toluidine derivative.	Neutral	Roach	1 in 500 turbid 1 in 1,000 solutions. I in 5,000	12-24 hrs. No effect. No effect.	Slight lemon yellow external staining. No internal staining.
19. Rosazurine G. Tolidine → methyl B naphthylamine delta sulphonic acid. + B naphthylamine delta sulphonic acid	Strongly alkaline	Roach	1 in 5,000 blood red solution with slight deposit.	24 hrs.	No external staining.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish.	Strength of Solution	Lethal Period	Remarks
20. Heliotrope. (dianisidine = di(methyl naphthylamine)	Neutral	Roach	1 in 1,000 deep purple solutions. No deposit.	No effect	Slight staining of fins and tail.
			1 in 5,000	No effect	No effect.
21. Ponceau R.R. (Beta naphthol disulphonic acid & diazo metaxylene.)	Neutral	Gudgeon	1 in 10,000 clear solutions but scum on surface	Within 24 hrs.	Slight external staining.
			1 in 100,000	do.	Marked internal staining especially of brain & spinal
			1 in 1,000,000	In 24 hrs.	Very slight staining. (cord.
22. Azo Phenyl Blue	Neutral	Gudgeon	1 in 10,000 slight suspension.	No effect	No effect.

TRI PHENYL METHANE COLOURS.

ROSANILIN GROUP.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution.	Lethal Period	Remarks.
1. Malachite Green. (Tetra methyl di amido tri phenyl carbinol.)		Roach & Gudgeon.	1 in 5,000 dark bluish green solutions	1½hrs.	In every fatal case much mucus was excreted which became deeply stained, as did tails & fins. There was also marked internal staining, especially of brains & spinal cords.
			1 in 25,000 clear solutions	1½ "	
			1 in 50,000	2 "	
			1 in 100,000	4 "	
			1 in 200,000 light colored solutions.	Within 12hrs.	
			1 in 1,000,000	No effect.	
2. Methyl Green. (Chlor methylate of hexa methyl para rosanilin chloride.)		Roach & Gudgeon.	1 in 10,000 solution clear and dark.	Within 24 hrs.	Much mucus excreted which was deeply stained. No other staining present.
			1 in 100,000 clear solution.	No effect.	No effect.
3. Rosanilin. (Tri amido tolyl di phenyl carbinol.)	Faintly alkaline	Gudgeon	1 in 10,000 formed clear solution very slowly.	Within 24 hrs.	Mucus excreted and stained. No internal staining
			1 in 100,000 clear solution	No effect.	No effect.
4. Rosanilin Acetate. (Most soluble rosanilin salt.)	Alkaline ?	Gudgeon.	1 in 10,000 deeply coloured. solutions.	Within 24 hrs	Slight external staining.
			1 in 100,000	No effect.	No internal staining.
5. Rosanilin Blue. (Gentian Blue) (Tri phenyl rosanilin chloride.	Neutral	Roach	1 in 5,000 half solutions	No effect	Unsatisfactory owing to insolubility.
			1 in 10,000 half suspensions.	No effect.	

Table of Experiments on Fish (cont.).

Substance.	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks
6. China Blue. (Sodium salt of di & tri sulphonic acids of triphenyl rosanilin & triphenyl para rosanilin.)	Neutral	Roach	1 in 1,000 very dark blue solution but clear.	Not fatal	Fish somewhat weak. Fins & tail brilliantly stained.
			1 in 5,000 also very dark	No effect	Died next day. No internal staining.
					No effect. Staining as above
7. Alkali Blue (Sodium salt of the monosulphonates of mono, di, & tri phenyl rosanilin & para rosanilin.	Neutral	Gudgeon	1 in 5,000 light coloured solution. Limit of solubility.	No effect	No effect.
8. Hofmann's Violet (Tri ethyl rosanilin.)	Neutral	Gudgeon.	1 in 10,000 } both dark clear	6 hrs.	Slight excretion of mucus, & slight external staining.
			1 in 100,000 } purple solutions	24 "	No internal staining.

TRI PHENYL METHANE COLOURS.

ROSOLIC ACID GROUP.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks
1. Rosolic Acid.	Acid	Roach	1 in 10,000 } both clear 1 in 100,000 } solutions.	3 hrs. Not fatal.	No staining. Fish rather weak.
2. Aurin or Yellow Corallin. (Sodium salt of a mixture of Aurin, Methylaurin, & Pseudo rosolic acid	Neutral or slightly acid	Roach	1 in 5,000 } clear, deep red solutions. 1 in 10,000 } 1 in 100,000 pale pink solution.	2½ hrs. Within 24 hrs. Not fatal	Very slight external staining. No internal staining. Fish very weak in 6 hours. No effect.

TRI PHENYL · METHANE COLOURS.

PHTHALEÏN GROUP.

Table of Experiments on Fish.

Substance.	Reaction	Species of Fish.	Strength of Solution	Lethal Period.	Remarks
1. Fluorescein. (Anhydride of tetra oxy di phenyl phthalide.	Neutral	Gudgeon	1 in 5,000 fluorescent solutions. 1 in 10,000 no suspended matter.	No effect No effect	No staining in either case.
2. Eosin. (Yellow Shade) (Tetra Brom fluorescein)	Acid	Roach & Gudgeon	1 in 5,000 solution 1 in 10,000 as above.	No effect.	No staining in either case

OXYQUINONE & KETONEIMIDE

GROUPS OF COLOURS.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks
I. OXYQUINONES.					
1. Alizarin (Di oxy anthra quinone)	Neutral	Roach & Gudgeon.	1 in 10,000 dark crimson solution with cloud at bottom.	Within 24 hrs.	No staining
			1 in 100,000 clear solution	No effect	
2. Purpurin. (Tri oxy anthra quinone)	Slightly acid.	Gudgeon	1 in 5,000 magenta coloured solution. Limit of solubility.	No effect	No staining.
II. KETONEIMIDES.					
1. Auramine. (Imide of tetra methyl di amido di phenyl methane hyhrochloride)	Neutral	Roach	1 in 5,000 all clear yellow solutions.	2½ hrs.	Brilliant & complete external staining. Marked internal staining, especially of brain & spinal cord. Fish shewed convulsive spasms.
		Minnow	1 in 10,000	6 "	
		Gudgeon	1 in 100,000	24 "	

QUINONEIMIDE COLOURS.

INDOPHENOL & OXYINDAMINE GROUPS.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks.
I. INDOPHENOLS.					
1, Methylene Blue. (Chloride of tetra methyl thionine.)	Slightly acid.	Roach & Gudgeon	1 in 10,000 } very dark blue 1 in 50,000 } solutions 1 in 100,000 lighter coloured.	4-6 hrs. No effect. No effect.	In fatal case there was slight external & internal staining, brain being coloured. Nasal pits deeply stained in all cases.
II. OXYINDAMINES.					
1. Gallo cyanin. (Chloride of di methyl phenyl ammonium di oxy phenoxazine carboxylic acid.)	Neutral	Roach	Colour obtained in solution 1 in 1,000 } both nearly 1 in 5,000 } colourless.	No effect No effect	No effect. No effect.

INDIGO AND QUINOLINE COLOURS.

Table of Experiments on Fish

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks
I. INDIGO COLOURS.					
1. Indigo Carmine (Indigotine di sulphonic acid)	Neutral	Roach	1 in 1,000 deeply colored solutions.	Not fatal	Fish somewhat weak. Slight external staining.
			1 in 5,000	No effect	No effect.
II. QUINOLINE COLOURS.					
1. Phosphine. (Mixture of nitrates of di amido phenyl acridine and of homologous bases	Neutral	Roach	1 in 5,000 dark, yellow solutions. Very slight suspension.	20 min.	Slight external staining.
			1 in 10,000	20 "	No internal staining.
			1 in 50,000 clear yellow solutions.	4 hrs.	External & internal staining. Brain & spinal cord specially coloured.
			1 in 100,000	4 "	
			1 in 1,000,000 very pale solution.	Not fatal	

AZINE AND INDULINE COLOURS.

Table of Experiments on Fish.

Substance	Reaction	Species of Fish	Strength of Solution	Lethal Period	Remarks.
I. AZINES.					
1. Saffranin (Para amido phenyl para amido phenazonium chloride)	Neutral	Gudgeon	1 in 10,000 dark red clear solution	Within 24 hrs.	Mucus excreted which was stained. No other staining.
			1 in 100,000 light red in colour	No effect	No effect.
2. New Blue. (chloride of di methyl phenyl ammonium B naphthoxazine.)	Neutral	Roach	1 in 5,000 } dark purple solutions, clear.	1 hr.	Great quantity of deeply stained mucus excreted.
			1 in 10,000 }	1 hr.	
			1 in 100,000 } faintly colored solutions.	6 hrs.	No internal staining.
			1 in 500,000 }	No effect	No effect.
II. INDULINES.					
1. Nigrosin. (Nitro compounds on anilin hydrochloride in presence of Ferrous Chloride).	Neutral	Gudgeon	1 in 10,000 violet blue, clear solution.	24 hrs.	Very slight staining of fins
			1 in 100,000 ditto	No effect.	No internal staining.

EXPERIMENTS WITH MORDANTS ON FISH.

Table of Experiments with Mordants on Fish.

Substance	Species of Fish	Strength of Solution	Lethal Period	Remarks.
1. Potassium Chromate.	Roach	1 in 1,000 clear yellow solutions 1 in 5,000 clear yellow solutions	30 hrs. No effect in 24 hrs.	Fish very weak in 24 hours, & shewed fungus growth.
2. Potassium Bichromate.	Roach	1 in 1,000	Within 24 hrs.	
3. Perchloride of Tin.	Roach	1 in 5,000 scum on surface and flocculent deposit. 1 in 10,000 scum on surface and flocculent deposit.	No effect in 24 hrs. do. do.	Probably unsatisfactory owing to action of calcium salts in Cambridge tap water.
4. Aluminium Nitrate.	Roach	1 in 5,000 clear solutions 1 in 10,000 clear solutions	No effect in 24 hrs. do. do.	
5. Chromium Sulphate.	Roach	1 in 10,000 ? some deposit as solution not complete. Also scum on surface.	No effect in 24 hrs.	
6. Oxalic Acid.	Roach	1 in 5,000 some white precipitate in both cases. 1 in 10,000 some white precipitate in both cases.	No effect in 24 hrs. do. do.	Unsatisfactory owing to formation of calcium oxalate.
7. Ferrous Sulphate.	Roach	1 in 10,000 copious reddish brown precipitate.	No effect in 24 hrs.	Unsatisfactory owing to precipitate.
8. Tartrate of Antimony.	Roach	1 in 5,000 clear solutions. 1 in 10,000 clear solutions.	32 hrs. No effect in 24 hrs.	Fish very weak in 24 hrs. & shewed fungus growth.

EXPERIMENTS ON FISH WITH MILL EFFLUENTS AND POLLUTED RIVER WATERS.

Experiments on Fish with Mill Effluents and Polluted River Waters.

Source	Contents, etc.	Species of Fish	Lethal Period	Remarks
Samples obtained from Tweed Mills and The River Tweed.	1. Claret coloured liquor containing Delta purpurin & Chrysophenine salt with Acetic Acid. Delta Purpurin = Tolidine / \ B naphthylamine delta monosulphonic acid. B naphthylamine delta monosulphonic acid. Chrysophenine = Di amido stilbene disulphonic acid di phenetol	Roach	4-6 hrs.	Solution was opaque but contained no suspended matter. Mucus secreted, and slight staining of fins and tail.
	2. Brownish black liquor containing Anthracene brown and Alizarin orange or hitro Alizarin.	Roach	4-6 hrs.	No staining, otherwise as above.
	3. Light green liquor containing Patent Blue, Acid Violet, and Bichromate of Potash. Patent Blue = Calcium salts of Sulphonic acids of meta oxy, meta amido or meta chlor tetra alkyl di amido triphenyl carbinol. Acid Violet. Sodium salt of penta methyl benzyl para rosanilin mono sulphonic acid.	Roach	2½ hrs.	Solution quite clear. No staining. Fish shewed signs of weakness in one hour.
	4. River water into which 1, 2, and 3 pass after precipitation, or filtration. Perfectly colorless	Roach	No effect in 24 hrs.	

Experiments on Fish with Mill Effluents & Polluted River Waters.

Source.		Contents, etc.	Species of Fish	Lethal Period	Remarks.
Samples obtained from polluted streams and rivers in Yorkshire.	I. 1.	Logwood Dyewater. Dark, odourless liquor, with black deposit, and slight acid reaction.	Gudgeon	3½ hrs.	No staining or clogging of gills. When deposit was removed by filtration through cotton wool, solution was not fatal in 24 hours, and had no apparent effect.
		Indigo Effluents.			
	II. 1.	From Hunsworth stream, Yorkshire. Fairly clear. No smell. Slight, dark deposit.	Roach	Not fatal.	No effect in 24 hours.
	2.	From Spen Beck stream, Yorkshire. Greenish coloured, with soapy smell.	Roach	Not fatal.	No effect.
	3.	From Dyeworks in Leeds. Dark blue solution, some deposit, very bad smell	Roach	Not fatal.	Fish at once much distressed. Great dyspnoea & weakness in 5 min. Gradually recovered and in a few hours quite lively and well.
	III. 1.	From River Aire. Liquor containing tannin and anilin dyes. Bluish black in colour. Soapy smell, faintly acid.	Roach	4 hrs.	Much mucus secreted.
	2.	From River Aire. Liquor containing chrome and anilin black. Much slimy deposit, No smell. Neutral.	Roach.	4 hrs.	Much mucus secreted.

Experiments on Fish with Mill Effluents & Polluted River Waters.

Source	Contents, etc.	Species of Fish	Lethal Period	Remarks.
Samples obtained from Leeds Dye Works.	1. Dark, odourless liquor faintly acid, taken from vat and containing Myrabolans and chlor nitrate of iron.	Roach	2 hrs.	Much mucus excreted.
	2. Logwood liquor from vat. Dark brown, odourless, no deposit.	Roach	3 hrs	" " "
Samples obtained from Mills and Streams in Lancashire.	1. Unpurified effluent (filtered) containing Copper Sulphate. Bichromate of Potash. Phosphate of Soda. Acetic Acid. Annatto. Sumach extract. Copperas. Pyrolignite of Iron. Logwood extract. Fustic. Yellow softening. Eagle cutch. Rasped Logwood.	Roach	About 3 hrs.	Reaction of effluent was slightly acid.
	2. Purified effluent. Purified by passage through Kremmolite, a form of iron ore.	Roach	Not fatal in 24 hrs.	Ditto. No effect.
	3. River water into which purified effluent had passed.	Roach	Not fatal in 24 hrs.	No effect.

Table of "single dose" Experiments on Rabbits.

Substance	Weight of Animal.	Dose & Method.	Symptoms	Lethal Period	Remarks
1. Azo Benzene	1,480 grm.	.37 grm. = $\frac{1}{4}$ grm. per kilo. body weight. Injected into stomach suspended in water and mucilage.	Nil	Nil	No effect save slight yellowness of conjunctivae.
2. Azo Benzene.	2,150 grm.	1.65grm. = about $\frac{3}{4}$ grm. p.kilo body weight. Injected as above.	Loss of appetite. Sluggishness. Weakness. Clonic & tonic spasms.	24 hrs.	Death due to acute enteritis and catarrhal & haemorrhagic nephritis.
3. Azo Benzene.	1,590 grm.	.8 grm. = $\frac{1}{2}$ grm. per kilo. body weight. Injected as above.	Do. Do.	40 hrs.	Do. Do. Do.
4. Azo Toluene	1,690 grm.	.38 grm. Injected as above	Loss of appetite. Slight weakness.	Nil.	No albuminuria or evidence of methaemoglobin.
Azo Colours.					
5. Anilin Yellow. (amido azo benzene.)	2,690 grm.	.68 grm. Injected as above.	Nil	Nil	No effect. Dye present in the urine.
6. Chrysoidine. (di amido azo benzene.)	1,650 grm.	.3 grm. Injected as above.	Nil	Nil	No effect. Dye present in the urine.

Table of "single dose" Experiments on Rabbits (cont.).

Substance	Weight of Animal	Dose and Method	Symptoms	Lethal Period	Remarks.
7. Bismark Brown. (Tri amido azo benzene)	1,760 grm.	.88grm.=½grm.p.kilo. body weight. Injected into stomach suspended in water & mucilage.	Nil	Nil	No effect. Dye present in the urine.
8. Brilliant Crocëin (Benzene azo benzene azo B naphthol disul-phonate of sodium.)	1,510 grm.	3 grm.= 2grm.p.kilo. body weight. Injected as above.	Nil	Nil	No effect. Dye present in the urine.
9. Helianthin (Benzene sul-phonate of sodium Azo di methyl anilin.	1,470 grm.	.74grm.=½grm.p.kilo. body weight. Injected as above.	Nil	Nil	No effect. Dye present in the urine.
10. Helianthin. (free base)	1,820 grm.	1.55grm.=⅚ grm.p.kilo. body weight. Injected as above.	Nil	Nil	No effect. Dye present in the urine.
11. Tropaeolin OO. (Benzene sul-phonate of sodium Azo di phenylamine.)	2,230 grm.	1.57grm.= about⅔ grm. p.kilo. body weight. Injected as above.	Nil	Nil	No effect save slight yellow-ness of conjunctivae. Dye not found in the urine.

Table of "single dose" Experiments on Rabbits (cont.).

Substance	Weight of Animal	Dose & Method	Symptoms	Lethal Period	Remarks
Nitro Colour.					
12. Aurantia (Sodium salt of hexa nitro di phenylamine.)	1,620 grm.	4,860 grm.=3grm.p.kilo. body weight. Injected into stomach suspended in water & mucilage.	Loss of Appetite. Weakness.	About 36 hrs.	No effect for 24 hrs. Post mortem shewed haemorrhages in stomach, congestion of large intestine & kidneys. Albumin & casts present in the urine.
Tri phenyl methane Colour					
13. Methyl Green. (Chlor methylate of hexa methyl para rosanilin chl.	1,340 grm.	2.7grm.= 2 grm.p.kilo. body weight. Injected as above.	Loss of Appetite. Weakness.	Nil	Animal rapidly became very weak, but soon entirely recovered.
Oxyquinone Colour.					
14. Alizarin. (Di oxy anthra quinone.	1,320 grm.	4 grm. = 3 grm.p.kilo. body weight. Injected as above.	Sluggishness.	Nil	No loss of appetite. Animal soon recovered. Dye present in urine.

Table of "long continued" Experiments on Rabbits.

Substance	Weight of Animal.	Dose & Method	Symptoms	Result	Remarks
Azo Colours.					
1. Anilin Yellow. (Amido azo benzene.)	2,290 grm.	1.14grm.= ½grm.per kilo. body weight, given daily in food from Feb.5-Mch.8.	Nil	Not fatal	No apparent effect. Dye present in urine. No albumin. Increase of weight.
2. Chrysoidine (Di amido azo benzene.)	2 kilogrm.	4 grm. = 2 grm. per kilo. body weight, given daily in food from Mch.2 - 24	Nil	Not fatal	No effect. No albumin.
3. Bismark Brown. (Tri amido azo benzene.)	2,300 grm.	4 grm.= About 2 grm.per kilo. body weight, given daily in food from March 10 to March 29.	Nil	Not fatal	Ditto.
4. Brilliant Orange. (Benzene azo B Naphthol sulphonate of Sodium.)	1,600 grm.	4.8 grm.= 3 grm.p. kilo. body weight given daily in food from July 12-25.	Nil	Not fatal	Only effect - diminution in urinary secretion. No albumin? Dye present in urine and faeces.
5. Tropaeolin 000 No. 2. (Benzene sulphonate of sodium azo B naphthol.)	1,850 grm.	2 grm. = 1 grm. per kilo. body weight, given daily in food from Feb. 15 to March 8.	Nil	Not fatal	No effect. No albumin. Dye present in urine.

Table of "long continued" Experiments on Rabbits (cont.).

Substance	Weight of Animal.	Dose & Method	Symptoms	Result	Remarks.
6. Orange G. (Benzene azo B naphthol di-sulphonate of sodium.)	1,500 grm.	4.5 grm.= 3 grm. p.kilo. body weight, given daily in food from July 12 to July 26.	Nil	Not fatal	No effect. No albumin. Dye present in urine & faeces.
7. Orange III (Nitro benzene azo B naphthol disulphonate of sodium.)	1,200 grm.	3.6 grm.= 3 gram p.kilo. body weight given daily in food from July 12 to July 19th.	Animal began to look ill in 2 days & refused food.	Not fatal	No albumin. Dye present in urine. Experiment stopped as rabbit would not eat.
8. Orange G.T. (Toluene azo B naphthol di-sulphonate of sodium.)	1 kilogrm.	3 grm.= 3 grm.p.kilo. body weight, given daily in food from July 23 - Aug.3.	Animal began to look ill in 4 days & refused food.	Animal chloroformed on August 3rd.	Movements became sluggish. No albumin or blood in urine, which contained the dye. No staining of tissues. Kidneys showed cloudy swelling.
9. Xylidène Red. (Xylene azo B naphthol di-sulphonate of sodium.)	950 grm.	3 grm.= about 3 grm.per kilo. body weight given daily in food from July 23 to August 4.	Diarrhoea after 6 days, followed by wasting. Food refused.	Found dead on Aug. 5.	No albumin found in urine, but dye present. Liver and kidneys congested. Latter showed cloudy swelling.
10. Cumidene Red. (Cumene azo B naphthol di-sulphonate of sodium.)	2,300 grm.	7 grm.= 3 grm.p.kilo. body weight, given daily in food from July 26 to August 3.	Food refused after 8 days. Animal looked distressed.	Not fatal.	No albumin in urine, but dye present. Experiment stopped.

Table of "long continued" Experiments on Rabbits (cont.).

Substance	Weight of Animal.	Dose & Method	Symptoms	Result	Remarks.
11. Fast Red B. (A naphthalene azo B naphthol disulphonate of sodium.)	1,550 grm.	4.7grm.= 3grm p.kilo. body weight, given daily in food from July 26 to August 3.	Food refused after 8 days. Animal looked distressed.	Not fatal	No albumin in urine but dye present. Experiment stopped.
12. Brilliant Crocëin. (Benzene azo benzene azo B naphthol disulphonate of sodium.)	2 kilogrm.	4 grm.= 2 grm.p.kilo. body weight, given daily in food from March 10 to March 27.	Nil	Not fatal	No effect. No albumin.
13. Helianthin (Benzene sulphonate of sodium azo di methyl anilin.)	1,400 grm.	4 grm.= 3 grm.per kilo. body weight, given daily in food from March 10 to March 28.	Nil	Not fatal	No effect. No albumin.
14. Tropaeolin 00. (Benzene sulphonate of sodium azo di phenylamine.)	1,500 grm.	3 grm.= about 2 grm.per kilo. body weight, given daily in food from Feb. 17 to March 8.	Food occasionally refused.	Not fatal	No effect. No albumin.

Table of Experiments on Rats by Subcutaneous Injection.

Substance	Nature of Colour.	Dose & Method	Symptoms	Lethal Period	Remarks
1. Chrysoidine. (Di amido azo benzene.)	Azo.	1 gr. dissolved in aq. dest., injected under skin of back.	Nil	Not fatal	No effect.
2. Brilliant Crocein. (Benzene azo benzene azo B naphthol di sulphonate of sodium.)	Azo.	Do.	Nil	Not fatal	No effect.
3. Congo Red. (Benzidine di naphthionic acid.)	Azo	Do.	Nil	Not fatal	No effect.
4. Ponceau R.R. (Beta naphthol disulphonic acid diazo metaxylene.	Azo.	Do.	Appearance of illness after 8 hrs. but food taken freely.	Between 12 & 24 hrs. Died during night.	Urine was of a bright crimson colour & contained dye. Post Mortem. Muscles, skin, intestines & all internal organs, except brain, stained red. Microscopic examination showed staining to be diffuse.
5. Malachite Green. (Tetra methyl di amido tri phenyl carbinol.	Triphenyl methane (Rosanilin)	Do.	Rapid onset of weakness. Dyspnoea.	35 min.	Post mortem. Brilliant staining of peritoneum & all abdominal organs. Muscles, heart & brain dark in colour, but not stained. ~~Lungs unstained.~~

Table of Experiments on Rats by Subcutaneous Injection (cont.).

Substance	Nature of Colour.	Dose & Method	Symptoms	Lethal Period.	Remarks.
6.Methyl Green. (Chlor methylate of hexa methyl para rosanilin chloride.)	Tri phenyl Methane (Rosanilin)	.1grm.dissolved in aq. dest., injected into back.	Marked weakness	2 hrs.	Post mortem. Congestion of liver & kidneys. Urine contained dye. No staining of muscles or internal organs.
7.Rosolic Acid.	Do. (Rosolic Acid group.)	Do.	Nil	Not fatal	No effect.
8.Eosin (Yellow Shade)	Do. (Pthalein group)	.1 grm. in suspension, injected as above.	Nil	Not fatal	No effect.
9.Alizarin. (Di oxy anthra quinone)	Di oxyquinone	.1 grm. dissolved in aq. dest., injected into back.	Nil	Not fatal	No effect.
10.Methylene Blue.	Quinoneimide (Indophenol)	Do.	Nil	Not fatal	No effect. Dye in urine.
11.Phosphine. (Mixture of nitrates of di amido phenyl acridine & of homologous bases.)	Quinoline.	Do.	Nil.	Not fatal.	No effect.

Table of Experiments on Rats by Subcutaneous Injection (cont.).

Substance	Nature of Colour.	Dose & Method	Symptoms	Lethal Period.	Remarks.
12. Saffranin. (Para amido phenyl para amido phenazonium chloride.)	Azine.	.1grm. dissolved in aq. dist. injected into back.	Marked weakness.	Not fatal	No effect.
13. Nigrosin. Nitro compounds on Anilin hydrochloride in presence of Ferrous chloride.	Induline.	Do.	Do.	Not fatal.	No effect.

A P P E N D I X III.

Tabulated List of Weyl's Experiments.

Table of Weyl's Experiments on Nitroso and Nitro Colours.

Dye	Animal	Dose	Symptoms	Result	Remarks
1.Di nitroso resorcinol.	Dog (a) 11,550 grm.	June 19th, 20th. 2 grm. daily. June 21. 3 grm	Nil.	Not fatal.	Traces of Albumin appeared in the urine, and there was slight loss of weight. Dye given by the stomach tube.
	Dog (b) 5,500 grm.	July 13. 1grm. " 15. 2grm. " 17. 3grm.	Nil.	Not fatal	Dye given by tube. Slight Albuminuria. Urine dark coloured.
	Dog (c) 5,250 grm.	July 4. 1grm. (subcutaneously)	Great weakness. Food refused.	Fatal in about 24 hours.	Dye injected under skin of back. Sulphates and Albumin in the urine. Post mortem, congestions and oedema of brain.
2.Naphthol Green B.	Dog (a) 5,800 grm.	May 15. 1 grm. " 16. 1 grm. " 17. 1grm. " 18. 5 grm.	Nil.	Not fatal.	Dye given by stomach tube. Conjunctiva stained intensely green. Urine greenish. Appetite not disturbed.
	Dog (b) 4,800 grm.	May 15. 2 grm. " 17. 2 grm.	Nil.	Not fatal.	Dye given by stomach tube. Urine and faeces green. No albumin. No loss of appetite.
	Dog (c). 5,650 grm.	May 24-28. 2 grm. daily. (subcutaneously)	Nil.	Not fatal.	Mucous membranes coloured green. No abscesses. No loss of appetite. Dog lively all the time.
	Dog (d). 5,015 grm.	May 25 - 28. 2 grm. daily. (subcutaneously)	Weakness, Fever. Abscesses.	Fatal on June 2nd.	Conjunctiva and mucous membranes coloured green 20 minutes after first injection. (cont.)

Table of Weyl's Experiments on Nitroso and Nitro Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
Naphthol Green B. (cont.)	Dog. (d). (cont.)				General effect first noticed on May 29. Abscesses on June 1. Post mortem. Serous membranes, liver and spleen green. In kidneys glomeruli not coloured, but uriniferous tubules green.
	Dog (e) 5,600 grm.	May 18 - 21 Amount not stated (subcutaneously)	As above.	Animal very weak, and killed on May 26.	As above. Rapid loss of weight.
3. Picric Acid as Sodium Picrate.	Dog. (very young)	April 21-26 .24 grm daily. Ap. 26-May 9 .36 grm daily. May 13 1.2 grm.	Nil.		Altogether the dog took the huge dose of 10.5 grm. in the course of 4 weeks, of which 9 grm of Sodium and Potassium Picrate had been given hypodermically. Even after getting 8.5 grm. dog was fairly well. Conjunctiva was tinged yellow. No post mortem record.
		May 14 .6 grm.	Weakness, Diarrhoea, Dyspnoea. Vomiting	Eventually fatal.	
		" " .36 "			
		" 15.24 "	Animal lively.		
		" " .72 "			
		" 16.16 "	Marked weakness & vomiting		
		" 17.72 "	Recovery (ing.		
		" 18.19 Nil.			
		" 20 1.32 grm.	Prostration.		

Table of Weyl's Experiments on Nitroso and Nitro Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks
4.Di Nitro cresol.	Rabbit (a) 890 grm.	.24 grm.	General	Fatal in 10 min.	1) 2) Laboratory Sample.
	Rabbit (b) 670 grm.	.08 "	Respirations soon became rapid. Animals became quiet, & sometimes fell to one side. Dragging of hind limbs.	Not fatal	3) 4) Sample from Dr Martius of 5) Berlin.
	Rabbit (c) 640 grm.	.16 "		Fatal in 15 min.	
	Rabbit (d) 670 grm.	.17 "		" " 21 "	6) Commercial Sample 7)
	Rabbit (e) 1,360 grm.	.34 "		" " 30 "	
	Rabbit (f) 1,880 grm.	.47 "	Muzzle touched ground & could not be raised. Pupils usually dilated. Twitchings and Spasms. Cheyne Stokes respiration. Asphyxia. Onset as a rule in 1 to 7 minutes.	" " 46 "	8) Commercial Sample. 9)
	Rabbit (g) 750 grm.	.187 "		" " 25 "	
	Rabbit (h) 680 grm.	.17 ? "		" " 143 "	10) Sample from human poison- 11) ing case. (Bremerhaven) 12)
	Rabbit (i) 1,797 grm	.45 "		" " 20 "	
	Rabbit (j) 1,610 grm.	.40 "		" " 120 "	13) Commercial Sample. 14)
	Rabbit (k) 1,970 grm.	.50 "		" " 165 "	
	Rabbit (l) 1,870 grm	.45 "		" " 135 "	In addition one rabbit (15) received hypodermic injections. 2 (Bremerhaven). Died in about 75 minutes with typical symptoms. Another (16) got .06 of same. Marked dyspnoea but no fatal result.
	Rabbit (m) 1,750 grm.	.42 "		" " 140 "	
	Rabbit (n) 1,690 grm.	.42 "		" " 150 "	

Table of Weyl's Experiments on Nitroso and Nitro Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks
Di Nitro cresol. (cont).	Dog (i) 6,230 grm.	1.5 grm. (By Stomach.)	General.	Not fatal.	1-7c - Laboratory Sample.
	Dog (ii) 5,500 grm.	.3 " (By Stomach.)	Dyspnoea. Vomiting.	Not Fatal.	8 - Bremerhaven Sample.
	Dog (iii) 5,500 grm.	.3 x" (By Stomach.)	Salivation. Diarrhoea.	x Fatal in 40 min.	9a.9b- Commercial Sample
	Dog (iv) 6,230 grm.	.3 " (By Stomach.)	Clonic spasms.	Not fatal	All commercial samples contain about 40% ammonium chloride.
	Dog (v) 14,000 grm.	.7 " (By Stomach.)	Tetanic spasms.	Not fatal	There was no methaemoglobin.
	Dog (vi) 6,230 grm.	0.1 x" (Hypodermically)	Often loud cries.	x Fatal in 75 min.	In fatal cases post mortem shewed hyperaemia of liver, intestines, and lungs.
	Dog (vii) 14,500 grm.a	0.1 " (Hypodermically)	Great weakness.	Not fatal	Toxic dose for dogs=7-10m.g (hypodermic) per kilo.
	" " b	0.1 " (Hypodermically)		Not fatal	Fatal dose for dogs=16 m.g.
	" " c	0.2 " (Hypodermically)		Not fatal	of pure salt. per kilo.
	Dog (viii) 3,420 grm.	0.1 x" (Hypodermically)		x Fatal in 170 min.	(potassium)
	Dog (ix) 5,690 grm.a	0.1 (Hypodermically)		Not fatal	
	" " b.	0.2 (Hypodermically)		Not fatal.	
5.Martius Yellow.	Dog (i) 6,850 grm.	Mch.10 .5grm.	Vomiting Diarrhoea Weakness	Fatal on Mch.15.	Some albuminuria. Great tendency to recovery during intermission. No p.m.
		" 12 .5 "			Given by Stomach.
		" 14 1 "			" " "
	Dog (ii) 5,700 grm.	1 "	Nil	Not fatal	

Table of Weyl's Experiments on Nitroso and Nitro Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
Martius Yellow (cont).	Dog (iii) 5,800 grm.	Ap.30, .1 grm. May 1, .1 " " 2, .1 " " 3, .1 " " 4, .15 " (all subcutaneously)	Continued Diarrhoea.	Not fatal.	Some albuminuria. Urine coloured.
	Dog (iv) 8,800 grm.	Ap.30 May 3. .1 grm. My. 4.2 " (all subcutaneously)	Strong dyspnoea Great thirst. Apathy.	Fatal on May 4.	Urine coloured. Contained albumin. Post mortem.- Venous congestion of internal organs.
6.Naphthol Yellow. S.	Dog (i) 4,800 grm.	May 7, 2 grm. " 8, 5.73 gr. " 11, 2 grm. " 12 2 grm.	Nil	Not fatal	Given by Stomach tube Extremely limited Albuminuria. Urine Coloured.
	Dog (ii) 5,800 grm.	" 11 .2 grm. " 13 .2 " (both subcutaneously)	Nil	Not Fatal	Animal lively. Nursed its pup. Appetite not impaired.
	Dog (iii) 1,040 grm.	May 11 .1 grm. " 13 .1 " (both subcutaneously)	Nil	Not fatal	Apparently no effect.

Table of Weyl's Experiments on Nitroso and Nitro Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
7. Brilliant Yellow.	Dog (i) 5,650 grm.	July 26 3 grm. " 28 3 " " 30 2½ " " 31 3 "	Nil	Not fatal	Urine coloured. Gained 180 grm. in weight by Aug. 5th
	Dog (ii) 11,600 grm.	July 28 ·2 grm. " 30 ·3 "	Nil	Not fatal	Gained 300 grm. in weight by August 5th.

Table of Wey'ls Experiments on Azo and Disazo Colours.

Dye	Animal	Dose	Symptoms	Result	Remarks.
Azo Colours					
1. Chrysoidin.	Dog (i) 26,600 grm.	Mch. 25 & 26 3 grm. Apl. 1 10 " " 6 10 " (by stomach)	Nil	Not fatal	No apparent effect. No vomiting. Urine originally contained albumin which did not increase.
	Dog (ii) 9,500 grm.	1 grm. daily for a month. (by stomach)	Nil	Not fatal	Animal lively, but lost about 1,200 grm (1/8 of body weight).
	Dog (iii) 58,500 grm.	Apl. 13 & 14 ·1 grm. " 29 & 30 ·1 " May 8 ·1 " Hypodermically	Nil	Not fatal	Animal lost about 1,230 grm. (1/5 body weight.)
	Dog (iv) 4,500 grm.	·1 grm. into abdominal cavity.	Nil	Not fatal	Slight albuminuria.
2. Bismark Brown.	Dog (i) 5,690 grm.	June 13 2 grm. " 14 2 " " 16 2 " " 22 5 "	Vomiting after each dose.	Not fatal	By stomach Slight albuminuria.
	Dog (ii) 29,500 grm.	Apl. 30 5 " May 2 5 " " 4 5 " " 8 15 " " 15 15 " (by stomach)	Slight Vomiting.	Not fatal	Dye present in urine. No albuminuria.

Table of Weyl's Experiments on Azo and Disazo Colours. (cont.)

Dye	Animal	Dose	Symptoms	Result	Remarks.
Bismark Brown. (cont.)	Dog (iii) 5,500 grm.	·25 grm. daily for a month. (in food)	Nil	Not fatal.	Animal gained 350 grm. in weight.
	Dog (iv) 6,300 grm.	9 injections ·1 grm. in 20 days.	Nil	Not fatal	No effect.
	Dog (v) 6,300 grm.	3 injections (abdominal) of ·1 grm.	Refused food. Slight fever.	Not fatal	Complete recovery in one week.
2. Soudan I.	Dog 11,900 grm.	June 8 2 grm. " 11 2 " " 13 2 " " 15 2 " " 23 5 " " 25 5 " (all by stomach)	Some vomiting and loss of appetite.	Not fatal	Varying albuminuria. Dye found in urine and faeces. No hypodermic injections as no suitable solvent.
3. Metanitra-zotin.	Dog (i) 12,600 grm.	July 12 1 grm. " 13 2 " " 14 2 " " 15 2 " (all by stomach)	Nil	Not fatal	150 grm. lost in five days.
	Dog (ii) 5,600 grm.	10 doses of 1 grm. in 2o days.	Nil	Not fatal.	No apparent effect.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
4. Paranitrazotin.	Rabbit. 1,500 grm.	2 doses of 2½ grm in 3 days.	Nil	Not fatal.	No effect.
5. Orange II or Tropaeolin 000.	Dog (i) 10,500 grm.	July 4 5 grm.	Urine coloured Vomiting and diarrhoea. Weakness and fever.	Fatal on July 23.	Much albumin in urine. Post mortem. Abscesses and fatty degeneration.
	Rabbit(ii) 2,250 grm.	3 grm.	None within four hours.	Death in 12 hrs.	No post mortem.
	Dog (iii) 4,300 grm.	Dec. 12 .5 " " 16 .25 " " 17 .5 " " 19 .5 " " 21 .75 " (all hypodermically)	Weakness. Diarrhoea Coryza Abscesses. Loss of hair.	Recovery after severe illness.	Dye present in urine. Albuminuria. 460 grm. lost in 15 days. Began to recover 5 days after administration stopped.
6. Ponceau 4G.B. or Brilliant Orange.	Dog (i) 12,400 grm.	June 19 2 " " 20 2 " " 21 2 " " 22 2 " " 23 4 " (all by stomach)	Nil	Not fatal	Urine coloured. No albumin. No effect.
	Dog (ii) 8,500 grm.	1 grm. daily for 1 month.	Nil	Not fatal	Slight loss of weight.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
7. Archil Substitute.	Dog (i) 3,810 grm.	Dec.27 & 28 1.64 grm. Dec.30 3.28 " Jan. 2 4.1 "	Nil	Not fatal	Apparently no effect. Urine Coloured.
	Dog (ii) 4,500 grm.	.82 grm. daily for 1 month= 24 grm.in all.	Nil	Not fatal	Urine not coloured.
	Dog (iii) 4,980 grm.	Jan.2 .82 grm. " 4 .82 " 9 1.64 " (all subcutaneously.)	Nil	Not fatal	No effects. Urine not coloured.
8. Diphenylamine Orange or Tropaeolin 00	Dog (i) 27,350 grm.	Jan.19 5 grm. " 21 5 " " 23 3 " " 28 10 " Feb. 2 15 " (all by stomach)	Nil	Not fatal	Some albuminuria. Slight loss of weight.
	Dog (ii) 9,730 grm.	Jan.19 3 " " 21 3 " " 23 2 " " 27 3 " " 28-30 3 " (daily) " 31 3 "	Nil	Not fatal.	Slight Albuminuria.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks
Diphenylamine (cont.)	Dog(iii) 5,450 grm.	Mch. 21 .1grm. " 24 .1 "" "31-Ap.4 .1 " (all subcutaneously.	Nil	Not fatal	No apparent effect.
9. Metanil Yellow.	Dog (i) 11,600grm.	Apl. 4 10grm. " 5 10 " (by stomach)	Vomiting. Dyspnoea. No food taken	Fatal on April 9th.	Post mortem. Stomach and liver contained unchanged colour.
	Dog (ii) 11,250grm.	Mch 21 1grm. " 26 10 " Apl. 1 5 " (by stomach)	Nil Vomiting. Little food taken.	Fatal on April 1st.	Post mortem. Yellow coloration of skin, subcutaneous tissue, serous membranes, etc. Liver congested, kidneys deep yellow.
	Dog (iii) 5,220 grm.	Apl. 6 .1 grm. " 9 .15 " " 11 .15 " (all subcutaneously)	Tremor.	Not fatal	Slight loss of weight.
10. Azarin S.	Dog (i) 25,600grm.	35grm.in 25 days (by stomach)	Nil	Not fatal.	Dye present in urine.
	Dog (ii) 10,300grm.	20grm.in 20 " (by stomach)	Nil	Not fatal	No apparent effect.
	Dog (iii) 4,700 grm.	3 injections of 1 grm.	Nil	Not fatal	" " " "
	Dog (iv)	5 c.c.of paste injected into abdominal cavity.	Weakness & loss of appetite.	Fatal.	Death from peritonitis. Red spots of dye substance in peritoneum.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
Disazo Colours	Dog (i) 9,630 grm.	Jan.19 3 grm. " 21 3 " " 23 2 " " 30 5 " Feb. 3 10 " (by stomach)	Diarrhoea. Loss of appetite	Not fatal	Animal quickly recovered when administrations stopped.
1. Fast Brown G.	Dog (ii) 5,900 grm.	2 grm. daily for 1 month. (by stomach)	Chronic diarrhoea. Loss of appetite.	Not fatal	Considerable loss of weight.
	Dog (iii) 6,730 grm.	Mch. 5 .1 grm. " 12 .1 " " 14 .1 " " 26 .1 " (all subcutaneously.	Nil	Not fatal	No apparent effect.
2. Wool Black.	Dog (i) 29,940 grm.	Dec.28 5 grm. " 29 5 " " 30 5 " Jan. 2 10 " " 4 10 " (all by stomach)	Nil	Not fatal	Dye present in urine. No apparent effect.
	Dog (ii) 3,500 grm.	Jan.21 .25 grm. " 23 .25 " " 24 .25 " " 28 .25 " (all subcutaneously)	Slight Abscess formation.	Not fatal.	No general effect.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
3. Naphthol Black. P.	Dog (i) 26,730grm.	Mch. 3 3 grm. " 4 3 " " 5 3 " " 6 5 " " 12 10 " " 14 20 " (by stomach)	Nil	Not fatal	Dye present in urine. Some loss of weight.
	Dog (ii) 4,500 grm.	1 grm. daily for 1 month.	Nil	Not fatal	No effect
	Dog(iii) 3,200 grm.	Mch. 5 .1 grm. " 7 .1 " (subcutaneously)	Loss of Appetite.	Fatal on March 8th.	Post mortem. Emaciation. Congestion of internal organs. No staining.
	Dog(iv)(weak) 3,860 grm.	Mch.12 .1 grm. " 13 .1 " " 14 .1 " (subcutaneously)	Animal very miserable. Great emaciation.	Killed Mch.25 as in dying state.	Post mortem. Subcutaneous cellular tissue and muscles stained. Liver congested and hypertrophied. Glands ditto.
4. Congo Red.	Dog (i) 7,300 grm.	34 grm. between Dec.18 & Dec.30	Slight loss of appetite.	Not fatal	No effect.
	Dog (ii) 4,300 grm.	1 grm.daily for 1 month.	Nil	Not fatal	" "
	Dog (iii) 4,970 grm.	Jan. 5 .25 grm. " 7 .25 " " 9 .25 " " 10 .25 " " 14 .25 "	Abscess formation. Weakness. Loss of appetite. Fever.	Recovery after severe illness.	Discharge from abscess contained Congo Red. Great loss of weight.

Table of Weyl's Experiments on Azo and Disazo Colours (cont.).

Dye	Animal	Dose	Symptoms	Result	Remarks.
5. Azo Blue.	Dog (i) 8,450 grm.	27 grm. between Jan.8 & Jan.15 at intervals of 2 - 3 days.	Nil	Not fatal	No apparent effect.
	Dog (ii) 4,700 grm.	1½ grm daily for 1 month.	Nil	Not fatal	" " "
	Dog (iii) 4,600 grm.	Jan.29 .20 grm. " 30 .20 " Feb.7 .20 " (all subcutaneously.)	Abscess formation	Not fatal.	Discharge from abscess contained unchanged Azo Blue.
6. Chrysamin R.	Dog (i) 9,230 grm.	Jan. 3 5 grm. " 4 5 " " 7 1 " " 9 1 " " 10 2 " " 11 2 " " 12 3 " " 15 3 " (by stomach)	Nil	Not fatal	Animal gained weight.
	Dog (ii) 11,300 grm.	21 grm. in 10 days.	Nil	Not fatal	Urine slightly coloured.
	Dog (iii) 3,680 grm.	Jan.10 .25 grm. " 11 .25 " " 12 .25 " " 15 .25 "	Abscess formation. Loss of appetite. Fever, melancholy.	Not fatal but experiment discontinued when animal very ill.	Abscesses contained gelatinous masses the colour of the dye. There was loss of weight.

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