THE TOXICITY TO ANIMALS OF 1:4 DIOXAN

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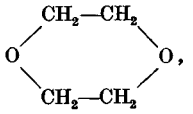
The material investigated was a mixture of 1 : 4 dioxan,



and water in the approximate ratio of 80 to 20, as these are the proportions of 1 : 4 dioxan and water in their constant boiling mixture, and are those employed in certain commercial processes. The mixture was carefully analysed and was found to have a constant boiling-point of 86·5° C. (uncorr.)—except for less than 2 per cent. of a higher boiling fraction, which eventually proved to be water—and a freezing-point of 2·1° C. It was free from ethylidine ethylene acetal, and from other impurities.

The physiological investigations were carried out under the following subheads:

A. Inhalation by animals.
   (*a*) Nominal concentration 1/100, 1 : 4 dioxan in air.
   (*b*) ,, 1/200, ,,
   (*c*) ,, 1/500, ,,
   (*d*) ,, 1/1000, ,,

B. Inhalation by human observers.
   (*a*) Nominal concentration 1/1000, 1 : 4 dioxan in air.
   (*b*) ,, 1/500, ,,

C. Intravenous injection—animals.

D. Feeding to animals.

E. Skin application—animals.

The technique and results of these experiments are now described.

A. Inhalation by animals

The selected animals were exposed in a one cubic metre chamber, and the desired concentration was obtained by vaporising the calculated quantity of the 1 : 4 dioxan water mixture.

In the case of the 1/1000 concentration vaporisation was obtained by heat; in all other concentrations by spray.

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In order to avoid condensation on the walls and also to avoid any effects from chill, the mean temperature of the chamber was maintained at about 27° C.

On five days in each week the animals received two 1½-hour exposures: on the sixth, one exposure was given, and the animals had a complete rest on the seventh.

(*a*) *Concentration* 1/100—*spray*

Six guinea-pigs, three rats and three mice were used.

All animals noticed the presence of something unusual at once, and rapidly displayed evidence of slight lachrymation. In all cases breathing was slightly distressed and this was more marked in the rats.

On opening the chamber after the first exposure, all animals seemed drowsy, but recovered rapidly. All three mice, guinea-pig 1 and rat 1 collapsed during the second exposure and were dead at the end of it. In most cases, collapse was preceded by convulsions. Guinea-pigs 2 and 3 died two hours later. Guinea-pigs 4 and 5 and rat 2 collapsed and died during the third exposure, and guinea-pig 6 was found dead after the fifth exposure. Rat 3, though obviously ill, survived seven exposures, and then died.

Morbid anatomy.

(*a*) *The lungs.* All the guinea-pigs and rats 1 and 3 showed pulmonary lesions that varied from an acute vascular congestion to an advanced infiltration of red blood corpuscles which might be described as "red hepatisation." The cause of death was a pulmonary one.

In rat 2 and the three mice the lung condition was less severe. To the naked eye, there appeared to be some degree of oedema, and the microscope showed an increased blood supply. It should be remembered that helminth infection of the lungs is commonly found in rats, and that entirely healthy lungs, in these animals, are unusual.

(*b*) *Kidneys.* In all animals, except rat 2, the kidneys showed evidence of serious damage. The changes were mainly cortical, and consisted in a marked patchy cell degeneration of the cortical tubules; the degeneration appeared to start as cloudy swelling of the cells. The other main features were intense vascular congestion, and haemorrhages, both inter- and intratubular. The medulla of the kidney in most cases escaped actual cell degeneration, but the haemorrhages, though less marked than in the cortex, appeared here as well.

The glomeruli were involved to a varying degree, sometimes escaping altogether, and sometimes severely damaged, either by haemorrhage into Bowman's capsule, or by cell degeneration.

(*c*) *Liver.* In all the guinea-pigs, in rat 3 and mice 1 and 2, the liver showed degrees of cell degeneration that varied from cloudy swelling to large

areas of complete necrosis. The lesions were patchy in their distribution, and appeared to have started at the periphery of the lobule.

In rats 1 and 2 the liver appearances were normal, and in mouse 3 the picture was one of vascular congestion only.

(*d*) *Other organs.* No abnormality noticed.

The cause of death.

In rat 1, the pulmonary condition was the only apparent cause of death.

In rat 3, and in all the guinea-pigs, the condition of the lungs was the *probable* cause of death. It will be seen, however, in the further experiments described below, that animals survived in apparently normal health, with even grosser lesions in both liver and kidneys.

In rat 2, and in the three mice, the renal condition was the main lesion and was the *probable* cause of death.

(*b*) *Concentration* 1/200—*spray*

The technique was identical with that employed in (*a*) above, with exposures of 1½ hours.

Four rabbits, six guinea-pigs, three rats and three mice were used, and the symptoms exhibited were comparable with those of that experiment.

Mouse 1 collapsed and died during the second exposure, as also did rat 1 after the sixth exposure. Rat 2 died during the ninth exposure and rat 3 after the tenth.

Guinea-pigs 1 and 2 were removed from the experiment after five and fourteen exposures respectively, owing to pregnancy which had not been noticed when the work was started. In each case the outcome was stillbirth. These two animals apparently recovered normal health, and were killed 42 and 36 days after their last exposure.

Mouse 2 was found dead shortly after the fifteenth exposure, and guinea-pig 3 and mouse 3 died after twenty-nine and thirty-four exposures respectively.

Of the remaining animals, which seemed to suffer very little inconvenience from repeated exposures, rabbits 2, 3 and 4 were killed after total exposures of 49½ hours each, and guinea-pigs 4, 5 and 6 after total exposures of 94½ hours. All the animals showed a progressive, but not excessive, loss of weight during the experiment.

The blood urea content of the rabbits was taken before exposure, and in Nos. 2, 3 and 4 was estimated at intervals until death.

Rabbit No.	Blood urea content, mgm. per 100 c.c.		
	Initial	7th day	23rd day (before being killed)
1	31	—	—
2	35	33	64
3	34	38	39
4	30	28	61

It is noticeable that in two rabbits the blood urea content was nearly doubled.

Morbid anatomy.

(*a*) *The lungs.* Rabbit 1 showed definite signs of broncho-pneumonia and probably died from this cause. In all the guinea-pigs and in rabbits 2, 3 and 4 the lungs were normal. The three rats showed a degree of vascular congestion in the lungs, of which, for reasons already stated, the significance is difficult to assess.

Mouse 1 died of pneumonia; mouse 2 showed some vascular congestion, and the lungs of mouse 3 appeared to be quite normal.

(*b*) *The kidneys.* All the rabbits, all the rats, and five out of the six guinea-pigs, showed well-marked degenerative changes, with haemorrhages in the renal cortex. In one of the rats the cortex of the kidney examined was practically destroyed.

One guinea-pig (No. 3) showed few changes beyond vascular congestion.

Two of the three mice also showed the same cell degeneration of the cortical tubules, while in the third the organ showed vascular congestion only.

Guinea-pigs 1 and 2 still showed well-marked lesions.

(*c*) *The liver.* Two rabbits, three guinea-pigs, three rats, and two mice showed fairly well-marked cell degeneration of the same type as in the previous experiment, starting at the periphery of the lobule, and with a patchy distribution. In the remaining animals venous distension was the only change, although in one rabbit (No. 4) this was so severe as to warrant the description of a red cell infiltration.

(*d*) *Other organs* all appeared to be normal.

Summary.

Rabbit 1 and mouse 1 probably died from pneumonia, although both animals showed well-marked lesions in both liver and kidneys. The rat lungs were—as usual—dubious. In the other animals the lungs were normal and the picture was one of hepatic and renal damage alone.

At this concentration, the likelihood of pulmonary affections, and death therefrom, appears to be much less than in the cases of animals exposed to 1/100.

(*c*) *Concentration* 1/500—*spray*

The technique was the same as in (*a*) and (*b*). Exposures of $1\frac{1}{2}$ hours, spaced as before, were given and four rabbits, four guinea-pigs, six rats and five mice were used.

The symptoms on exposure were slight, and the animals appeared to suffer little inconvenience.

Rabbit 2 died after exposures totalling 69 hours, and the other animals were killed for examination as follows:

	Total exposure hours
Rabbit 1	45
Guinea-pig 1. Rat 1	48
Rabbits 3, 4	99
Guinea-pigs 2, 3, 4. Rats 2, 3, 4, 5, 6. Mice 1, 2, 3, 4, 5	102

In all animals the weight either increased slightly or remained steady.

Blood urea content in the rabbits.

Before death, rabbits 1, 3 and 4 had blood urea contents as follows:

Rabbit 1. 32 mg. urea per 100 c.c. blood
,, 3. 28 ,, ,,
,, 4. 27 ,, ,,

which are all within normal limits. (The blood urea content was investigated in a series of fifty normal rabbits. The highest reading was 46 mg. per 100 c.c. of blood and the lowest 26 mg., with an average of 35·02 mg. The estimations were carried out by the method described by Maclean in *Modern Methods in the Diagnosis and Treatment of Renal Disease* (2nd ed., London, 1924).)

Post-mortem examination of urine found in the bladder

Rabbit No.	Albumen	Casts	Leucocytes	Epithelial cells	Phosphatic deposit
1	Nil	None	None	None	+
3	Trace	Many granular	Few	Few	+
4	Minute trace	Few granular	Few	None	+

Morbid anatomy.

Rabbit 2 (which died after 69 hours' exposure) showed a very advanced degree of degeneration and necrosis in the cortical tubules of the kidney. There were many haemorrhages—large and small—scattered about in the cortex and streaking the medulla. The liver showed patchy cell degeneration of the usual type. The lungs and other organs were healthy and it is fair to conclude that the renal and hepatic lesions caused death. In the remaining animals the lesions may be summarised as follows.

(*a*) *The kidneys.* Rabbit 1 and guinea-pigs 1 and 4 showed severe lesions in the kidney comparable both in degree and in type with those found in rabbit 2.

In rabbits 3 and 4, guinea-pigs 2 and 3, and rat 1, the renal lesions were less severe, but cell degeneration was still well marked, and accompanied by haemorrhages.

In rats 2, 3, 4 and 6 and mouse 5, no definite cell degeneration was noted, but there was very marked vascular congestion, associated with haemorrhages throughout the kidney.

In rat 5 and mice 1, 2, 3 and 4, no lesion beyond varying degrees of vascular congestion was discernible.

(*b*) *The liver.* Rabbit 1, guinea-pigs 1 and 4 and rat 1, all showed cell degeneration of the usual type, with marked vascular congestion. In rabbit 3, guinea-pigs 2 and 3 and rats 2, 3 and 4, vascular congestion was the only lesion, but in rats 2 and 6 this feature was so extreme that it amounted to a red cell infiltration. In rabbit 4, rat 5, and all the mice the liver appeared to be normal.

(*c*) No lesions were noted in the lungs or in other organs.

With the inhalation of a 1/500 concentration, therefore, the incidence of pulmonary inflammation had ceased, and the renal and hepatic lesions were the only ones observed.

It must be noted that this particular series of exposures was started later than those involving inhalation of 1/1000. The total exposures were therefore less and, on the whole, the lesions in the kidneys and liver were less severe.

(*d*) *Concentration* 1/1000—*put up by heat*

The technique was identical with that employed in experiments (*a*), (*b*) and (*c*), except that the 1 : 4 dioxan-water mixture was vaporised by heat. The exposures were each of the same length and were spaced as before.

The animals employed were two rabbits, three guinea-pigs, three rats and four mice.

The visible symptoms were naturally much less severe than in the higher concentrations employed previously.

They amounted to little more than evidence of slight discomfort, and an appearance of noticing something strange. The rabbits still took up their characteristic defensive attitude, but this and other symptoms tended to lessen in the latter part of the several exposures.

Morbid anatomy.

Mouse 1 died from accidental causes after eight 1½-hour exposures (12 hours in all). The only change noted was an extreme vascular congestion of the liver, which amounted to a red blood cell infiltration. The remaining animals were all killed at intervals and their total exposure varied from 78 to 202½ hours. Their apparent health remained unaffected, and their weight either remained stationary or slightly increased. In rabbit 1 the increase in weight was greater, but an undiscovered pregnancy was the cause of this.

(*a*) *The lungs* were normal in all animals.

(*b*) *The kidneys.* With the exception of mouse 1, all the animals showed well-marked lesions in the renal cortex. These were of the same type that has already been described and in some cases were of extreme severity.

(*c*) *The liver.* Only rat 2 had a normal liver. In guinea-pig 3 and mice 1, 3 and 4, the changes were limited to a well-marked vascular congestion. All the other animals showed definite cell degeneration, which varied from patches of cloudy swelling to large areas of complete necrosis.

(*d*) *Other organs.* No abnormality was noted.

Observation on the blood urea content (rabbits). This was not taken at the beginning of the experiment:

Rabbit No.	Blood urea, mgm. per 100 c.c.	
	After 6 weeks	After 12 weeks (at death)
1	28	46
2	32	28

the readings being within normal limits.

Summary.

Pulmonary inflammation does not occur at this concentration, and the lesions were exclusively hepatic and renal.

B. Inhalation by human observers

(*a*) *Concentration* 1/1000—*nominal*

This concentration was obtained by vaporising the 1 : 4 dioxan-water mixture in a ten cubic metre chamber.

Four observers entered and remained in the chamber for five minutes.

Detection was immediate, as a rather sickly odour, and one observer stated that he experienced a sense of constriction in the throat and a desire to breathe more quickly: in fact, he thought that his respiratory rate was slightly increased. The other observers noted nothing beyond a not unpleasant sensation of warmth in the throat and chest, which rapidly faded.

(*b*) *Concentration* 1/500—*nominal*

This concentration was obtained by the same method and in the same chamber.

Six observers entered and remained for three minutes.

The atmosphere had a strong ethereal or spirituous odour.

There was no lachrymation and no desire to cough. It was noticeable that the strong smell appeared to diminish rapidly during exposure, and, on entering the chamber again, a few minutes later, the smell was much less noticeable. There was nothing in either of these atmospheres to cause the least alarm or discomfort, and, as stated, the very mild symptoms produced at first, rapidly diminished.

It is therefore considered that concentrations of this order (1/1000 or 1/500) could readily be tolerated by workers for prolonged periods, without any subjective sensations that would act as a warning.

That these concentrations are capable of producing severe renal and hepatic lesions in animals has been shown in the preceding work. As far as it is permissible to argue from animal to man, they would presumably be very definitely dangerous to human workers.

C. EFFECTS OF INTRAVENOUS INJECTIONS

Nine rabbits were used in all.

In the first series of four rabbits, each animal received a single injection of 1 : 4 dioxan-water mixture into the lateral ear vein as follows:

Rabbit No.	80 % 1 : 4 dioxan c.c.	Normal saline c.c.
1	1	9
2	2	8
3	3	7
4	5	5

each injection totalling 10 c.c.

One control animal received 10 c.c. of saline which produced no effect whatever. All four struggled violently after the injection of the first few *drops. The animal which received a 5 c.c. dose went into convulsions and* collapsed immediately after the injection, but recovered rapidly.

All the animals appeared to be in normal health the next day and, except for a loss of weight of from 4 to 8 oz. in the next fortnight, remained so, until they were killed for examination a month after the injections were given.

Microscopically, no abnormality was found post-mortem. The bladders of all contained urine which was examined with the following results:

Rabbit No.	Albumen	Casts	Renal epithelial cells	Phosphates
1	Trace	Many granular	Yes	—
2	,,	Few granular	,,	—
3	,,	,,	,,	Loaded
4	Minute trace	None	,,	—

Microscopical morbid anatomy

Kidney. All animals showed the same type of cortical degeneration with haemorrhages that has been described before. In No. 3 this was particularly advanced and the degenerative changes extended into the medulla. All four showed, in varying degree, a clear contrast between healthy and diseased tissue.

Liver. In Nos. 1 and 4 no abnormality was noted in the liver sections. No. 2 showed areas of cloudy swelling, and No. 3 extensive and gross cell degeneration, starting as before at the periphery of the lobules.

No abnormality was found in other organs.

The second intravenous experiment was carried out on three rabbits, Nos. 5, 6 and 7.

Each received two doses, with an interval of 48 hours, of 5 c.c. 1 : 4 dioxan-water mixture with 5 c.c. normal saline.

The immediate effects were the same as before, struggling, convulsions, and collapse, followed by a rapid return to normal. Five days after the first injection No. 7 became acutely ill, and was killed. Two days later Nos. 5 and 6 also became ill: No. 5 was killed and No. 6 died.

Morbid anatomy

(*a*) *Macroscopic.*

In all three the kidneys were enlarged. This was most definite in No. 7 whose kidneys exceeded the normal size by 50 per cent. The liver was normal in No. 6, mottled in No. 5 and pale in No. 7.

(*b*) *Microscopic.*

Kidney. In all three the renal cortex was practically destroyed, a few glomeruli standing out on a background of necrotic tubules in a mesh-work of connective tissue. The medullary tubules had escaped degeneration, but many were blocked with blood casts and hyaline material. Haemorrhages were frequent both in cortex and medulla.

Liver. In all cases there were extensive degenerative changes in the hepatic cells, again apparently starting from the edges of the lobules.

The third experiment was carried out on rabbits Nos. 8 and 9 in order to study the effect of intravenous 1 : 4 dioxan on the blood picture, and the blood urea content.

In this case each animal received 4 c.c. of pure 1 : 4 dioxan mixed with an equal quantity of normal saline. The pure compound was prepared from a redistilled sample of dihydroxyethyl ether, supplied by Messrs British Drug Houses, Ltd. The B.P. of the ether was 123·5°/14 mm. and had D_{15}^{14} 1·124. The 1 : 4 dioxan obtained from this was purified by refluxing with aqueous sodium hydrate and separated by salting out with anhydrous potassium carbonate. The pure product boiled at 100·5°/765 mm., froze at 10·9° C. and had D_{15}^{15} 1·041.

The immediate symptoms were the same as those already noted after a 5 c.c. dose of the 1 : 4 dioxan-water mixture.

Twenty-four hours after injection both animals developed a hind-limb paralysis. In No. 8 this was partial and bilateral, in No. 9 complete and confined to the left hind-leg.

This showed no improvement and, on humanitarian grounds, both animals were killed seven days after the injection.

The pathological results of the experiments were as follows:

(*a*) *The blood picture*

	No. 8	No. 9
	(1) Before the injection	
Leucocyte count	11,600	12,400
Red cell count	4,150,000	4,870,000
Haemoglobin	80 %	80 %
Abnormal cells	None	None
	(2) 24 hours after injection	
Leucocyte count	34,200	16,000
Red cell count	2,490,000	3,170,000
Haemoglobin	65 %	70 %
Abnormal cells	None	None
	(3) 48 hours after injection	
Leucocyte count	13,200	14,400
Red cell count	3,760,000	4,610,000
Haemoglobin	65 %	70 %
Abnormal cells	None	None

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It will be noted that the immediate changes were a diminished red cell count, diminished haemoglobin, and an increased white cell count, and that there was a rapid return to normal limits in the number of cells:

(*b*) *Blood urea content in mg. per* 100 *c.c.*

(1) Before injection		(2) 24 hours after injection		(3) Day before destruction	
No.	Blood urea	No.	Blood urea	No.	Blood urea
8	31	8	38	8	37
9	38	9	40	9	36

the variation being within normal limits.

(*c*) *Morbid anatomy*

(1) *Macroscopic.*

In both animals, beyond a moderate kidney enlargement, no abnormality was noted.

(2) *Microscopic.*

Kidney. Both animals showed the same picture, namely, advanced cell degeneration of the cortical tubules with many haemorrhages, and blockage of the medullary tubules with blood casts.

Liver. No. 8 showed a well-marked cell degeneration of the familiar type, while in No. 9 no definite changes were detected.

D. FEEDING EXPERIMENTS

Six rats and six mice were used, and were supplied daily with water containing 5 per cent., by volume, of the 1 : 4 dioxan-water mixture to an excess above their normal requirements. No other liquid was given to them.

After a few days the rats appeared to be slightly more quiet than before the experiment, which may have been merely a naturally increasing docility. This was not noticed in the mice, which were very tame from the start.

On the 11th day mouse 1, which appeared to be in normal health, was accidentally killed. Rat 1, which had been ailing for about 48 hours, died on the 14th day, and rats 2 and 3 were found to be *in extremis*, and were killed on the 19th day. Rats 4 and 5 became ill and died on the 31st and 34th day respectively.

Mice 2 and 3 were killed for examination on the 60th day and rat 6 and mice 4, 5 and 6 on the 67th day. These six animals appeared to be in normal health when they were killed.

Morbid anatomy

(*a*) *Macroscopic.*

Rat 1 had a heavy helminth infection of the lungs. In rats 1, 2, 3, 4 and 5 the kidneys were much enlarged; this was especially marked in Nos. 2 and 3, in which the kidneys were about twice the normal size. Rats 4 and 5 showed signs of an acute gastro-enteritis. Mouse 1 showed the usual appearances of

death from asphyxia. In mice 2 and 3 there was a definite enlargement of both kidneys and spleen. In rat 6 and mice 4, 5 and 6 the naked-eye appearances were normal.

(*b*) *Microscopic.*

(1) *The kidneys.* All the rats and mice 1, 2 and 3 showed severe lesions in the renal cortex, of the familiar patchy type. Cell degeneration was very advanced, and had, in most cases, reached the stage of large necrotic areas. In the medulla, as in the other experiments, cell degeneration was slight or absent, but many tubules were blocked with casts. Haemorrhages and vascular congestion were marked features throughout the kidney.

In mouse 5, the cortical cell degeneration was in an earlier stage, and had not proceeded beyond a cloudy swelling. In mice 4 and 6 cell changes had apparently not begun, but in 4, 5 and 6, vascular congestion was very marked and haemorrhages were present in both cortex and medulla.

(2) *The liver.* Cell degeneration—of the same type—again occurred in five rats and three mice. This was extreme in rats 2 and 5, well marked in rat 6, and in an early stage in rats 1 and 3, and mice 1, 2 and 3. In all these animals vascular congestion was well marked in addition, and appeared to be the only abnormality in rat 4 and mice 4, 5 and 6.

(3) *The spleen.* In mouse 2 the spleen showed areas which took up the stain (haemalum) poorly, but there were no gross changes. In mouse 3 the spleen was normal in appearance.

Summary.

The rats in the experiment seemed to be more severely affected than the mice, and two rats died with an acute enteritis. The renal and hepatic tissue changes were also more severe in the rats.

E. Skin application

As a guide to the lipoid and aqueous solubility of 1 : 4 dioxan, its partition coefficient between toluene and water was estimated and found to be in the proportion of 1/0·7. It was therefore anticipated that dioxan would be readily absorbed through the skin.

Four rabbits and four guinea-pigs were used, and an area was clipped free from fur on the nape of each animal: the smallest abrasion was avoided.

Each rabbit received ten drops and each guinea-pig five drops of the 1 : 4 dioxan-water mixture upon the clipped area eleven times in each week. The applications were spaced as follows: two applications daily on five days in each week, one application on the sixth and a blank day on the seventh.

No skin irritation whatsoever resulted from these applications.

The animals remained in apparently normal health, and were killed for

examination in pairs (one rabbit and one guinea-pig) at intervals as shown below:

Animal No.	Day killed	Total No. of applications	Weight 1st day lb.	Weight 1st day oz.	Weight day of death lb.	Weight day of death oz.
Rabbit 1	49th	75	5	0	4	12
„ 2	66th	105	4	8	4	10
„ 3	77th	121	4	12	4	14
„ 4	101st	160	4	8	4	12
Guinea-pig 1	49th	75	1	6	1	8
„ 2	66th	105	1	6	1	4
„ 3	77th	121	1	8	1	4
„ 4	101st	160	1	6	1	8

Morbid anatomy

Macroscopically nothing definitely abnormal was found in any of the eight animals, but the microscopical findings in the sections of the liver and kidneys are of sufficient interest to deserve a separate description for each animal.

Rabbit 1.

Kidney cortex. Widespread areas of cloudy swelling in cells of the tubules; glomeruli appear rather full. Blood vessels are dilated, and a few scattered haemorrhages can be seen.

Kidney medulla. Normal.

Liver. Normal.

Rabbit 2.

Kidney cortex. Extensive degeneration of tubule cells, which in places has reached the stage of necrosis. This degeneration involves the glomeruli as well. There are frequent haemorrhages.

Kidney medulla. Cells are normal: streaky haemorrhages occur.

Liver. Well-marked patchy cell degeneration, which is most obvious at the periphery of lobules.

Rabbit 3.

Kidney cortex. Extreme degree of almost generalised necrosis, with glomeruli standing out, darkly stained against a background of pale degenerated tubules. Many haemorrhages.

Kidney medulla. Streaky haemorrhages.

Liver. Vascular congestion. Well-marked patchy cell degeneration.

Rabbit 4.

Kidney cortex. Well-marked cloudy swelling which is just short of definite cell degeneration, vascular congestion extreme.

Kidney medulla. Normal.

Liver. Vascular congestion only.

Guinea-pig 1.

Kidney. No actual cell degeneration observed. Vascular congestion and a few haemorrhages both in cortex and medulla.

Liver. Normal.

Guinea-pig 2.

Kidney cortex. Well-marked patchy degeneration. Haemorrhages present.

Kidney medulla. Some tubules are blocked with blood casts.

Liver. Patches of early cloudy swelling of cells.

Guinea-pig 3.

Kidney cortex. Gross degeneration and necrosis of cortical tubules. Glomeruli swollen and often obliterated. Haemorrhages are very marked.

Kidney medulla. Many streaky haemorrhages.

Liver. Sections were not obtained from the liver of this animal.

Guinea-pig 4.

Kidney cortex. Well-marked patchy cloudy swelling, which in places is passing to the stage of definite cell degeneration. No haemorrhages seen.

Kidney medulla. A few tubules are blocked with casts.

Liver. Early patchy cell degeneration.

It will be noticed that the renal and hepatic lesions become progressively worse in the first three pairs of animals killed, rabbit 1 and guinea-pig 1 (49th day) showing early, rabbit 2 and guinea-pig 2 (66th day) advanced, and rabbit 3 and guinea-pig 3 (77th day) gross tissue changes. Curiously enough, in the fourth pair (killed on the 101st day) the kidney and liver appearances are intermediate in degree of severity between the first and second pairs, and yet the sections do not suggest recovery or repair.

In rabbits 3 and 4 the blood urea content on the days of their deaths was 29 and 28 mgm. per 100 c.c. respectively. The urine of rabbit 3—taken post-mortem from a distended bladder—was viscid and opaque with phosphates, but showed no albumen and, on centrifuging, no casts.

It is of interest that an animal—such as rabbit 3—can live in apparently perfect health, put on two ounces in weight, and show a normal blood urea content, with the cortices of its kidneys practically destroyed.

DISCUSSION OF RESULTS

1. Experiments have been conducted in which eighty-eight animals (twenty-three rabbits, twenty-three guinea-pigs, twenty-one rats and twenty-one mice) have been subjected to the effects of 1 : 4 dioxan.

Effects have been produced by inhalation (nominal concentrations 1/100, 1/200, 1/500 and 1/1000) by intravenous injections, by feeding and by application to the clipped skin.

2. The outstanding results have been severe lesions in the kidneys and the liver of the animals employed.

3. Of the eighty-eight animals, only one (rat 1 in series A (*a*)) escaped with no damage to either kidneys or liver, presumably dying from pneumonia before the renal and hepatic lesions had time to develop.

4. The remaining eighty-seven animals all suffered definite damage either in the kidneys or the liver. In the majority, both organs were affected, and the lesions were severe in degree.

5. The inhalation effects of 1 : 4 dioxan upon guinea-pigs have been investigated by Yant, Schrenk, Waite and Patty (1930). The pathological effects described by these authors are irritative signs in the respiratory tract, congestion and oedema of the lungs and a hyperaemia of the vessels of the surface of the brain—renal and hepatic lesions, as observed in the present series, are not recorded by them. Also, 1 : 4 dioxan was one of the compounds investigated by Van Oettingen and Jirouch (1931) in their study of the pharmacology of ethylene glycol and its derivatives. They noted the occurrence of acute nephrosis, marked filling of the intracapsular spaces and renal tubules with blood, and other degenerative changes in rats, following subcutaneous injections.

6. Human observers have been subjected to short inhalation exposures of nominal concentrations of 1/500 and 1/1000, both of which strengths have been proved, in the experiments described above, to be toxic to animals.

All the observers agreed that there was nothing in these concentrations to warn or alarm an uninstructed individual. Moreover, such slight effects as these concentrations produced at first, rapidly wore off, and it is considered that they would produce no subjective symptoms that would render prolonged exposure intolerable or even unpleasant.

7. The experiments dealing with inhalation and skin absorption have a very obvious practical interest from the industrial standpoint.

Conclusions

1. 1 : 4 dioxan produces severe toxic effects upon animals, whether it is administered by inhalation, intravenously, orally, or by skin application.

2. The most striking pathological feature is the progressive character of the damage to the kidney and liver.

3. The respiratory tract is only affected when very high concentrations are inhaled.

Summary of inhalation experiments

Animal	Total exposure hours	D. = died K. = killed	Main pathological features
Concentration = 1/100			
Guinea-pig 1	3	D.	P.K.L.
„ 2	3	D.	K.L.P.
„ 3	3	D.	P.K.L.
„ 4	4·5	D.	K.L.P.
„ 5	4·5	D.	P.K.L.
„ 6	7·5	D.	K.L.P.
Rat 1	3	D.	P.
„ 2	4·5	D.	K.
„ 3	10·5	D.	K.L.P.
Mouse 1	3	D.	K.L.
„ 2	3	D.	K.L.
„ 3	3	D.	K.
Concentration = 1/200			
Guinea-pig 1	7·5	K.	K.L.
„ 2	21	K.	K.L.
„ 3	43·5	D.	L.K.
„ 4	94·5	K.	K.L.
„ 5	94·5	K.	K.L.
„ 6	94·5	K.	K.L.
Rat 1	9	D.	K.L.P.
„ 2	13·5	D.	K.L.P.
„ 3	15	D.	K.L.P.
Mouse 1	3	D.	P.K.L.
„ 2	22·5	D.	P.K.L.
„ 3	51	D.	L.K.
Rabbit 1	16·5	D.	K.L.P.
„ 2	49·5	K.	K.L.
„ 3	49·5	K.	K.L.
„ 4	49·5	K.	K.L.

Animal	Total exposure hours	D. = died K. = killed	Main pathological features
Concentration = 1/500			
Rabbit 1	45	K.	K.L.
„ 2	69	D.	K.L.
„ 3	99	K.	K.
„ 4	99	K.	K.
Guinea-pig 1	48	K.	K.L.
„ 2	102	K.	K.L.
„ 3	102	K.	K.L.
„ 4	102	K.	K.L.
Rat 1	48	K.	K.L.
„ 2	102	K.	K.L.
„ 3	102	K.	K.L.
„ 4	102	K.	K.
„ 5	102	K.	K.
„ 6	102	K.	K.L.
Mouse 1	102	K.	K.
„ 2	102	K.	K.
„ 3	102	K.	K.
„ 4	102	K.	K.
„ 5	102	K.	K.
Concentration = 1/1000			
Rabbit 1	144	K.	K.
„ 2	196·5	K.	K.
Guinea-pig 1	106·5	K.	K.L.
„ 2	147	K.	K.L.
„ 3	202·5	K.	K.L.
Rat 1	78	K.	K.L.
„ 2	147	K.	K.
„ 3	202·5	K.	K.L.
Mouse 1	12	K.	L.
„ 2	106·5	K.	L.K.
„ 3	147	K.	P.K.L.
„ 4	202·5	K.	K.L.

Note. In column 4 for each concentration: K. = kidney, L. = liver, P. = lung.

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Summary of intravenous injection, feeding and skin application experiments

Intravenous injection

Animal	Dosage	Interval between doses hours	Day after 1st dose died (D.) or killed for exam. (K.)	Main pathological features
Rabbit 1	1 c.c. 80 %	—	K. 29th	K.
„ 2	2 c.c. 80 %	—	K. 29th	K.L.
„ 3	3 c.c. 80 %	—	K. 29th	K. + L. +
„ 4	5 c.c. 80 %	—	K. 29th	K.
„ 5	5 + 5 c.c. 80 %	48	K. 7th	K. + + L. +
„ 6	5 + 5 c.c. 80 %	48	D. 7th	K. + + L. +
„ 7	5 + 5 c.c. 80 %	48	K. 5th	K. + + L. +
„ 8	4 c.c. pure	—	K. 7th	K. + + L. +
„ 9	4 c.c. pure	—	K. 7th	K. +

Feeding—5 % in water

Animal	Day from start died (D.) or killed for exam. (K.)	Main pathological features
Rat 1	D. 14th	K. + L.
„ 2	K. 19th	K. + L. +
„ 3	K. 19th	K. + + L.
„ 4	D. 31st	K. + L.
„ 5	D. 34th	K. + L.
„ 6	K. 67th	K. + L. +
Mouse 1	D. 11th	K. + L.
„ 2	K. 60th	K. + L.
„ 3	K. 60th	K. + L.
„ 4	K. 67th	K.L.
„ 5	K. 67th	K.
„ 6	K. 67th	K.

Skin application. Rabbits 10 drops and guinea-pigs 5 drops twice daily

Animal	Day killed for exam. (K.)	Main pathological features
Rabbit 1	49th	K.
„ 2	66th	K. + L.
„ 3	77th	K. + + L. +
„ 4	101st	K.
Guinea-pig 1	49th	K.
„ 2	66th	K.L.
„ 3	77th	K. + + +
„ 4	101st	K.L.

Note. In the last column of each section K. = kidney, L. = liver.

REFERENCES

VAN OETTINGEN, W. F. and JIROUCH, E. A. (1931). *J. Pharmacol. and Exp. Therap.* **42**, 355.

YANT, W. P., SCHRENK, H. H., WAITE, C. P. and PATTY, F. A. (1930). *Public Health Report (U.S. Pub. Health Service)*, **45**, 35, 2023–2032.

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