

Report on toxicity test of carbonyl sulfide

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Abstract

COS sample was provided by Chengdu Grain Storage Research Institute. Its toxicity has been studied in the four phases in routine testing procedure. The results showed that COS was a low toxic pesticide. $LC_{50} > 2000\text{mg/m}^3$, no irritation to derma and eyes in acute tests to mice and rats. All tests of mutagenesis employed reasonably showed negative results. Abnormal indexes including viscera coefficient, hemogram and biochemical index were very few, mostly in male rats in the 90-days test in rats who were fed by the fumigated died of COS at the dosage of 50, 100, 200 and 500g/m^3 , which indicated that maximal effect less level (MNL) was 20g/m^3 . In the tests of teratogenesis to rats, the abnormal index was only average weight of fetus, showing that MNL was 50g/m^3 . Rats of two-generation test of reproduction in F_0 and F_1 generations, MNL was 50g/m^3 . The results in two-year feeding test and test of carcinogenesis to rats showed that some biochemical indexes in different period were abnormal, indicating MNL was 20g/m^3 , but no special tissue injury and higher incidence of tumor were discovered.

In conclusion, COS was regarded as a low toxic and low hazardous fumigant and its MNL was 20g/m^3 .

Part One Acute Toxicity Test, Skin and Eye Stimulating Test

Methods

Animal: twenty adult S D rats, half male and half female
twenty adult mice, half male and half female
rats' back were clipped free of hair (area $4 \times 5\text{cm}^2$)

Exposure route: static inhalation exposure for 14 days
COS dose level: $2\text{g/m}^3/2\text{hours/d}$

Indicates: death rate, manifestation
rats' derma and eye reaction

Result

All rats and mice lived normally, and no death was

observed

No injury or irritation was observed on the eye or derma.
COS was low toxic $LC_{50} > 2\text{g/m}^3$.

Part Two Mutagenesis Test

Includes: Ames test; E coli reversion mutation test; Mice marrow red cell micronucleus test; Moce chromosome aberration test.

Ames test

Methods

- Strains: Salmonella typhimurium of histidine auxotroph TA97, TA98, TA100, TA102.
- Groups: Negative control group: administrated with distilled water
Positive control group: administrated with 4QNO, 2-AF
Four test groups: COS dose level: 1, 5, 10 and 50g/m^3 respectively
- Procedure: Test was carried out according to air exposure way referred by Maron Ames in 1983. Mammalian S₉ activation system was added or not added. Triplicate plates per dose per strain was used in each assay

Results

The counts of reversion bacterial colony of positive control group was two times more than that of negative control group no matter with or not.

The counts of reversion bacterial colony of four test groups did not exceed two times that of negative control group in spite of with or not

The result showed that mutagenesis effect was not observed in COS under the condition of given dose level

Escherichia coli reversion mutation test

Methods

- Tester strains: Escherichia coli of tryptophan auxotroph, WP₂, WP_{2UVRA}, CMR891.
Escherichia coli of lactose and VB₂ auxotroph, ND₁₆₀, MR₂₋₁₀₂.
- Groups:

A negative control group: administrated with distilled water

A positive control group: administrated with 4QNO or 2-AF

Four test groups: COS dose level: 50, 100, 500 and 1000mg/m^3 respectively.

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• Procedure:

Test was carried out with air exposure way referred by Green in 1984. Mammalian activation system was added or not added.

Triplicate plates per dose per strain was used in each assay

Results

Revertant bacterial colony count of positive control group

was two times more than that of negative control group in spite of with activation system or not

Revertant bacterial colony count of four test groups did not exceed two times that of negative control group no matter with activation system or not

The result showed that mutagenesis effect was not observed in COS under the condition of given dose level in Escherichia coli reversion mutation test

Table 1. Ames test result ($\bar{x} \pm s$).

Group	concentration of COS(g/m ³)	S9	Revertant bacterial colony counts			
			TA97	TA98	TA100	TA102
Negative						
Control	0	-	132.3 ± 10.7	32.4 ± 2.9	142.8 ± 17.5	271.0 ± 29.2
COS	1	-	134.5 ± 10.3	34.6 ± 7.1	142.2 ± 18.2	266.3 ± 26.1
	5	-	125.0 ± 10.3	37.1 ± 7.2	144.5 ± 11.8	269.3 ± 30.9
	10	-	137.1 ± 36.3	28.6 ± 5.9	131.7 ± 18.0	256.5 ± 4.5
	50	-	73.0 ± 13.1	11.7 ± 6.5	49.3 ± 13.5	100.6 ± 36.9
	4QNO	0.5ug/plate	-	909.6 ± 48.9	362 ± 34	1042 ± 58
Negative						
Control		+	163.5 ± 10.0	51.8 ± 10.8	166.1 ± 11.7	281.6 ± 30.2
COS	1	+	158.8 ± 13.1	52.3 ± 10.8	171.0 ± 26.6	289.5 ± 28.8
	5	+	170.5 ± 12.1	55.3 ± 10.8	171.0 ± 26.6	289.5 ± 28.8
	10	+	155.4 ± 18.4	45.8 ± 6.9	168.0 ± 11.8	338.6 ± 21.9
	50	+	125.6 ± 18.1	18.5 ± 1.5	103.5 ± 4.5	214.3 ± 17.5
2-AF	50ug/plate	+	1973 ± 98.8	1530 ± 230	1596 ± 56	419 ± 56

Table 2. Escherichia coli reversion mutation test ($\bar{x} \pm s$)

Concentration of COS (mg/m ³ 2hr)	WP2		WP2uvrA		CMR ₈₉₁		ND ₁₆₀		MR ₂₋₁₀₂	
	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
50	16 ± 4	14 ± 2	16 ± 2	20 ± 9	30 ± 7	31 ± 8	6 ± 4	5 ± 3	3 ± 2	5 ± 1
100	19 ± 6	10 ± 3	15 ± 6	20 ± 5	33 ± 4	34 ± 6	4 ± 2	4 ± 2	5 ± 1	5 ± 1
500	17 ± 6	13 ± 4	16 ± 6	26 ± 4	32 ± 6	34 ± 9	4 ± 3	6 ± 2	7 ± 3	6 ± 1
1000	18 ± 5	10 ± 2	19 ± 7	22 ± 8	40 ± 10	35 ± 7	6 ± 3	6 ± 4	5 ± 4	5 ± 3
Negative control group	19 ± 4	10 ± 2	19 ± 7	22 ± 8	40 ± 10	31 ± 8	4 ± 2	5 ± 1	6 ± 3	6 ± 2
Positive control group	105 ± 6	78 ± 13	>600	>200	>600	>200	>100	52 ± 7	>100	43 ± 10

Mice marrow red cell micronucleus test

Methods

•Respiratory route

Animal: mice, weight 25 – 30g, were distributed into six groups randomly.

Groups: A negative control group: administrated with distilled water.

A positive control group: administrated with cyclophosphamide (CP).

Four test groups: COS dose level 0.1, 0.5, 1.0 and 2.0g/

m³ respectively.

Procedure: Mice of four test groups were exposed twice to COS gas of corresponding dose level via respiratory route on the first and twenty-fourth hour. Each exposure lasted two hours. Mice of positive group were administrated with 40mg/kg CP via a single intraperitoneal injection. The animals were killed after 30 hours dosing. Marrow was performed as routine method

Indicate: Calculate rate of micronucleus among every one thousand polychromatic erythrocyte (PCE)

•Oral route

Animal: Mice, weight 25 – 30g, were distributed into six groups randomly.

Groups: A negative control group: administrated with distilled water.

A positive control group: Administrated with CP

Four test groups: Administrated with COS diluted in cooking plant oil Dose level was 5mg/kg, 10mg/kg, 50mg/kg and 100mg/kg respectively.

Test Procedure: Mice of four test groups were administrated twice with COS diluted in plant oil of corresponding dose level via oral route on the first and the twenty-fourth hour. Mice of positive group were administrated with 40mg/kg CP via a single intraperitoneal injection The animals were killed after 30 hours dosing Marrow was performed as routine method.

Indication: Calculate rate of micronucleated cell among every one thousand polychromatic erythrocytes(PCE)

Results

There were no differences of micronucleated cell rate between the four test groups and the negative control group no matter via respiratory route or oral route

The results showed that micronucleus test of COS was negative.

Table 3. Micronucleus test of COS via respiratory route ($\bar{x} \pm s$).

Concentration of COS (g/m ³ 2hrs)	Number of mice	Number of cell examined	Rate of micronucleus (%)
0.1	10	10000	2.2 ± 0.84
0.5	10	10000	1.6 ± 0.89
1.0	10	10000	1.6 ± 1.44
2.0	10	10000	2.0 ± 1.22
Negative control group	10	10000	2.4 ± 0.89
Positive control group	10	10000	17.4 ± 3.05*

Compared with the negative control group * P < 0.01

Table 4. Micronucleus test of COS via oral route ($\bar{x} \pm s$)

Concentration of COS (mg/kg)	Number of mice	Number of cell examined	Rate of micronucleus (%)
5	10	10000	2.0 ± 0.71
10	10	10000	1.8 ± 0.84
50	10	10000	1.6 ± 0.55
100	10	10000	2.2 ± 0.45
Negative control group	10	10000	2.0 ± 0.71
Positive control group	10	10000	18.6 ± 4.04*

Compared with the negative control group * P < 0.01

Mice chromosome aberration test

Methods

•Inhalation route

Animal: Male mice, weight 25 – 30g, were distributed into five groups randomly.

Groups: A negative control group: administrated with distilled water.

A positive control group: administrated with CP.

Three test groups: exposed to COS gas, dose level 0.25g/m³, 0.5g/m³ and 1.0 g/m³, respectively.

Procedure: Mice of three test groups were exposed to COS gas of corresponding dose level two hours each day via respiratory route for five days continuously. Mice of positive group were administrated with 40mg/kg CP one time each day via oral route for five days continuously. . Animals were killed 13 days later Mice were administrated with 5mg/kg colchicine via intraperitoneal injection six hours before animals were killed. Samples of primary spermatocytes were performed as routine method.

Indication: Calculate the frequencies of chromosome aberration every one hundred primary spermatocytes.

•Oral route

Animal: Mice, weight 25 – 30g, were assigned to six groups randomly

Groups: A negative control group: administrated with distilled water

A positive control group: administrated with CP.

Three test groups: administrated with COS diluted in cooking plant oil Dose level was 5mg/kg, 10mg/kg, 50mg/kg and 100mg/kg respectively

Procedure: Mice of three test groups were administrated with COS diluted in plant oil of corresponding dose level via oral route once a day Mice of positive group were administrated with 40mg/kg CP via a single intraperitoneal injection. Animals were killed 13 days later. Mice were administrated with 5mg/kg colchicine via intraperitoneal injection six hours before animals were killed.

Samples of primary spermatocytes were performed as routine method.

Indication: Calculate the frequencies of chromosome aberration every one hundred primary spermatocytes.

Results

The frequencies of chromosomal aberration, X – Y univalent and autosomal univalent in mice primary spermatocytes in three test groups of two exposure routes did not show difference from those of negative control group.

The results showed that COS has no genotoxicity in germ cells of mice.

Table 5. Mice chromosome alteration test of COS via inhalation route ($\bar{x} \pm s$).

Concentration Of COS (g/m ³ .2hr)	counts of spermatocytes	X - Y univalent	autosomal univalent	type of aberration			rate of aberration
				break	triradial	quadriradial	
0.25	500	4.4 ± 0.84	0.4 ± 0.55	4	0	0	0.8 ± 0.45
0.5	500	4.0 ± 1.22	0.6 ± 0.55	4	0	0	0.8 ± 0.45
1.0	500	4.4 ± 1.14	0.6 ± 0.55	3	0	0	0.6 ± 0.55
negative control group	500	4.2 ± 0.84	0.6 ± 0.55	3	0	0	0.6 ± 0.55
positive control group	500	10.2 ± 1.30*	6.8 ± 0.84*	61	13	29	20.6 ± 5.13*

Compared with the negative control group * P < 0.01

Table 6. Mice chromosome alteration test of COS via oral route ($\bar{x} \pm s$).

Concentration Of COS (mg/kg)	counts of spermatocytes	X - Y univalent	autosomal univalent	type of aberration			rate of aberration
				break	triradial	quadriradial	
25	500	4.4 ± 0.55	0.8 ± 0.45	4	0	0	0.8 ± 0.45
50	500	4.2 ± 0.45	0.6 ± 0.55	2	0	0	0.4 ± 0.45
100	500	4.0 ± 0.71	0.6 ± 0.55	3	0	0	0.6 ± 0.55
negative control group	500	4.2 ± 1.10	0.6 ± 0.55	2	0	0	0.4 ± 0.55
positive control group	500	10.2 ± 1.30*	6.8 ± 0.84*	61	13	29	20.6 ± 5.13*

Compared with the negative control group * P < 0.01

Part Three Subchronic Toxicity Study

Methods

Animal: S. D rats They were assigned to six groups randomly, half male, half female in each group.

Groups: a negative control group, five test groups.

Procedure: Weaning rats of the five test groups had been fed with the basal diet fumigated with gaseous COS of corresponding dose for 90 days, basal diet fumigated without aeration. The doses were 20g/m³, 50g/m³, 100g/m³ and 500g/m³ respectively.

The animals of negative control group were fed with normal basal diet. The animals of the five groups were killed 90 days later.

Indication: body weight increase, food consumption rate, hematology, biochemical measures, pathological examination.

Results

Body weight increase

Body weight increase of the five test groups had no difference from that of the control group.

Food consumption rate

Food consumption rate of the other test groups had no difference from that of the control group except that food consumption rate of the sixth group of female was significantly higher than that of the control group.

Table 7. Animal body weight increase ($\bar{x} \pm s$).

Groups	Concentration of COS (g/m ³)	body weight increase of male rats (g)	body weight increase of female rats (g)
1	0	211.1 ± 35.52	139.3 ± 19.99
2	20	205.8 ± 42.73	137.2 ± 19.99
3	50	226.9 ± 36.27	129.7 ± 17.75
4	100	202.7 ± 36.27	138.6 ± 28.50
5	200	232.6 ± 46.96	129.9 ± 40.97
6	500	224.8 ± 33.76	140.6 ± 30.89

No statistical difference compared with the control group

Table 8. Animal food consumption rate ($\bar{x} \pm s$)

groups	Concentration of COS (g/m ³)	food consumption rate of male	food consumption rate of female
1	0	17.08 ± 2.10	13.05 ± 1.84
2	20	16.34 ± 2.43	13.60 ± 2.30
3	50	18.75 ± 1.68	15.36 ± 2.99
4	100	16.62 ± 2.05	14.11 ± 1.97
5	200	17.69 ± 2.01	13.72 ± 1.91
6	500	16.52 ± 2.14	16.55 ± 2.97*

Compared with the negative control group * P < 0.01

Organ coefficient

Organ coefficients of the other test groups had no difference from those of the control group except that teste/

body of the fourth and the fifth groups of male were significantly higher than those of the control group. No pathological abnormality was observed in the main organs

Table 9. Animals' organ coefficients ($\bar{x} \pm s$)

sex	group	Concentration of COS (g/m ³)	liver/body	renal/body	spleen/body	teste/body
male	1	0	3.65 ± 0.27	0.74 ± 0.05	0.21 ± 0.04	0.90 ± 0.11
	2	20	3.68 ± 0.23	0.72 ± 0.05	0.23 ± 0.04	0.97 ± 0.16
	3	50	3.60 ± 0.25	0.69 ± 0.04	0.22 ± 0.03	0.90 ± 0.07
	4	100	3.81 ± 0.50	0.71 ± 0.14	0.24 ± 0.06	1.01 ± 0.07*
	5	200	3.79 ± 0.35	0.76 ± 0.06	0.23 ± 0.03	1.03 ± 0.08*
	6	500	3.52 ± 0.57	0.75 ± 0.10	0.26 ± 0.06	0.94 ± 0.12
female	1	0	3.85 ± 0.50	0.79 ± 0.10	0.28 ± 0.07	
	2	20	3.75 ± 0.20	0.77 ± 0.04	0.27 ± 0.05	
	3	50	3.59 ± 0.39	0.73 ± 0.08	0.27 ± 0.05	
	4	100	3.55 ± 0.38	0.74 ± 0.06	0.28 ± 0.07	
	5	200	3.44 ± 0.30	0.70 ± 0.06	0.27 ± 0.05	
	6	500	3.55 ± 0.27	0.71 ± 0.06	0.29 ± 0.05	

Compared with the male control group * $P < 0.05$

Hematology

White blood cell (WBC) of the third and the fifth groups of male were significantly lower than that of the control group.

Neutrophil of the fifth and the sixth groups of males were

significantly lower than that of the control group

Lymphocyte of the fifth and the sixth groups of male were significantly higher than that of the control group.

WBC of the fifth group of female was significantly lower than that of the control group.

Table 10. Hematology examination ($\bar{x} \pm s$).

sex	group	Concentration of COS (g/m ³)	RBC (× 10 ¹² /L)	WBC (× 10 ⁹ /L)	neutrophil (%)	lymphocyte (%)	Hb (g/L)
male	1	0	6.75 ± 0.88	10.95 ± 3.61	14.9 ± 5.3	85.0 ± 5.1	169.2 ± 8.5
	2	20	7.55 ± 1.12	9.11 ± 3.07	14.7 ± 4.5	85.3 ± 4.5	162.5 ± 8.9
	3	50	7.46 ± 0.82	7.79 ± 2.18*	13.3 ± 3.1	86.6 ± 2.9	169.3 ± 22.3
	4	100	7.09 ± 0.80	8.89 ± 1.97	11.9 ± 3.4	87.9 ± 3.4	173.1 ± 10.4
	5	200	6.45 ± 0.50	6.46 ± 1.58*	9.8 ± 3.5*	90.2 ± 3.6*	168.7 ± 7.8
	6	500	7.07 ± 0.96	9.21 ± 1.77	7.7 ± 3.7*	92.1 ± 3.6*	173.5 ± 5.9
female	1	0	6.09 ± 0.57	9.91 ± 2.87	11.5 ± 1.9	88.3 ± 1.8	167.9 ± 11.2
	2	20	6.49 ± 0.77	8.01 ± 2.26	12.7 ± 3.7	86.8 ± 3.8	173.8 ± 7.4
	3	50	6.38 ± 0.99	7.92 ± 2.74	12.4 ± 3.4	87.6 ± 3.4	165.9 ± 12.3
	4	100	6.50 ± 0.74	8.05 ± 1.55	11.0 ± 3.2	88.9 ± 3.1	157.2 ± 10.5
	5	200	6.93 ± 0.82	6.81 ± 1.23*	10.3 ± 4.7	89.4 ± 5.2	165.3 ± 10.4
	6	500	6.56 ± 0.86	7.50 ± 2.04	12.0 ± 2.3	88.3 ± 2.6	154.4 ± 5.3

Compared with the same sex control group * $P < 0.05$

Biochemical measurement

ALB of the third, the fourth, the fifth and the sixth groups

of male were significantly higher than those of the control group.

Table 11. Biochemical measurements ($\bar{x} \pm s$).

sex	group	Concentration Of COS (g/m ³)	GPT (u/L)	ALB (g/L)	GIO (g/L)	BUN (mmol/L)
male	1	0	129.9 ± 11.75	38.99 ± 2.27	32.3 ± 4.6	9.42 ± 1.76
	2	20	120.0 ± 9.24	39.51 ± 1.89	33.4 ± 2.9	10.0 ± 2.99
	3	50	124.4 ± 18.52	41.12 ± 2.02*	31.7 ± 3.8	9.35 ± 1.53
	4	100	121.1 ± 17.37	42.98 ± 2.46*	32.1 ± 5.6	9.15 ± 1.26
	5	200	105.6 ± 10.45	41.26 ± 1.74*	32.9 ± 3.6	8.23 ± 1.12
	6	500	120.3 ± 16.85	43.40 ± 3.66*	30.3 ± 5.0	8.12 ± 0.82
female	1	0	112.6 ± 19.90	45.23 ± 3.02	27.1 ± 5.5	8.16 ± 1.21
	2	20	112.9 ± 20.65	47.01 ± 3.56	27.5 ± 5.8	8.44 ± 0.86
	3	50	114.2 ± 12.36	49.11 ± 4.80	31.5 ± 5.4	7.86 ± 1.00
	4	100	93.0 ± 8.42	46.04 ± 2.97	26.2 ± 4.4	8.54 ± 0.97
	5	200	93.8 ± 10.44	47.84 ± 2.49	28.2 ± 6.9	8.73 ± 1.97
	6	500	93.2 ± 14.19	44.91 ± 3.59	28.3 ± 5.6	8.33 ± 0.93

Compared with the same sex control group * P < 0.05

The results of subchronic studies showed;

1. All the indication of 20g/m³ COS fumigating group showed no abnormalities.
2. Some indication of 50g/m³, 100g/m³, 200g/m³ and 500g/m³ COS fumigating group showed abnormalities, especially in the male groups. 20g/m³ COS fumigating level would be considered as the maximal no effect level (MNL)

Part Four Teratology Test

Methods

Traditional teratology test

Animal: The pregnant rats were assigned to five groups

Groups: a negative control group, administrated with distilled water; a positive control group, administrated with CP; three test groups

Procedure: Pregnant rats had been fed with the basal diet fumigated with COS gas. The last day before birth, the rats were killed to examine the fetus

Feeding teratology test

Animal: The weaning rats were distributed into seven groups

Group: a negative control group, a positive control group, three test groups.

Procedure: Weaning male and female rats of five test groups had been fed with the basal diet fumigated with COS gas of corresponding dose for 90 days. Males and females within a given treatment group were mated to produce F1a generation. The last day before birth, the rats were killed to examine the fetus. In both methods the animals of positive control group were administrated with Dikozuo known as a strong teratogen via oral route. The animals of negative control group were fed with normal basal diet.

Results

Traditional teratology test

Fetus survival: Average weight of fetus in three test groups had no statistical difference with the negative control group. Rate of live fetus and resorption were almost equal to that of the negative control group. No early dead fetus in the three test groups was observed.

Table 12. Fetus survival ($\bar{x} \pm s$).

Group	Concentration of COS (g/m ³)	number of pregnancy	number of implication	rate of live fetus (%)	rate of resorption	rate of early dead fetuses	average weight
1*	0	12	125	95.2	4.8	0	3.6 ± 0.4
2*		10	69	21.7	72.5	5.8	2.4 ± 0.6
3	100	12	135	97.0	2.9	0	3.5 ± 0.4
4	200	12	127	95.3	4.7	0	3.5 ± 0.4
5	500	9	103	98.1	1.9	0	3.7 ± 0.4

* note: 1. negative control group

2. positive control group

Organ and body abnormality: No genitals and gross abnormalities in the three test groups were observed. observed in the three test groups and negative control group.

Skeletal abnormality: No skeletal abnormality was

Table 13. Results of gross and genitals abnormality.

Group	Concentration Of COS (g/m ³)	rate of cranium abnormality(%)	rate of limb abnormality(%)	rate of tail abnormality(%)	rate of organ abnormality(%)
1*		0	0	0	0
2*		100	60	60	20
3	100	0	0	0	0
4	200	0	0	0	0
5	500	0	0	0	0

* note: 1. negative control group
2. positive control group

Table 14. The rate of skeletal abnormality.

Group	Concentration of COS (g/m ³)	rate of sternum abnormality(%)	rate of rib abnormality(%)	rate of coccyx abnormality(%)	rate of spinal abnormality(%)	rate of abnormality(%)
1*	0	0	0	0	0	
2*		10	10	20	10	50
3	100	0	0	0	0	0
4	200	0	0	0	0	0
5	500	0	0	0	0	0

* note: 1. negative control group
2. positive control group

Feeding teratology test

Average weight of fetus in fifth, sixth and seventh groups were lower statistically than the negative control group.

The seventh group (500g/m³) had 3.0% resorption and 3.0% early dead fetus.

Rate of live fetus was almost equal to that of the negative control group.

Implication and live fetus rate in the test groups were almost equal to the negative control group.

COS of 20g/m³ and 50g/m³ had no teratogenic effect.

Table 15. Fetus survival ($\bar{x} \pm s$).

Group	concentration of COS (g/m ³)	number of pregnancy	number of implication	Rate of live fetus (%)	rate of resorption	rate of early dead fetuses	average weight
1	0	11	125	96.8	3.2	0	4.0±0.7
2		10	69	21.7	72.5	5.8	2.4±0.6*
3	20	10	83	98.9	0	0	4.3±1.0
4	50	11	99	94.9	0	0	3.7±0.2
5	100	11	91	100	0	0	3.3±0.4*
6	200	11	91	100	0	0	3.4±0.3*
7	500	11	100	94	3.0	3.0	3.4±0.4*

note: 1. negative control group
2. positive control group

* compared with the negative control group $P < 0.05$

Part Five Generation Test

Methods

Groups: a negative control group; five test groups.

Test procedure: Weaning rats fed with the basal diet fumigated with COS gas were distributed into six groups. After 100 days dosing, males and females (F₀) within a given treatment group were mated to produce F1a generation. F1a generation continued to produce F2a generation with the above methods of F₀.

Animals of negative control group were fed with normal

basal diet.

Results

Mating rate, pregnancy rate and rate of pups when born in the five test groups of F₀ and F₁ generation had no difference from the control group.

Rate of pups alive when weaning in the sixth group of F₀ generation was lower than that of control group.

Rates of pups alive when weaning in the fourth, the fifth and the sixth groups of F1a generation were lower than that of control group. It showed that COS could cause lactating rats lack of milk when fumigating concentration was higher than 100g/m³.

Table 16. Two generation test.

generation	group	Concentration of COS (g/m ³)	number of mating	number of gestation	Mating rate(%)	Pregnancy rate(%)	pups rate alive when born(%)	rate of pups alive when weaning
F ₀	1	0	22	151	68.2	100	86.2	96.8
	2	20	22	18	81.8	100	98.1	93.4
	3	50	20	16	80.0	100	96.0	93.3
	4	100	20	14	70.0	100	89.0	95.0
	5	200	22	18	81.3	100	92.0	91.3
	6	500	24	18	75.0	100	69.9	79.8*
F1a	1	20	24	21	87.5	100	96.9	80.2
	2	50	24	18	75.0	100	92.9	85.3
	3	100	24	20	83.3	100	85.5	83.6
	4	200	24	19	79.0	100	92.2	32.0*
	5	500	24	24	100	100	89.9	36.5*
	6		24	18	75.0	100	90.5	55.3*

note: 1 negative control group

* compared with the negative control group $P < 0.05$

Part Six Chronic Toxic Test and Carcinogenesis Test

Chronic Toxic Test

Methods

Animal: Weaning rats were distributed into six groups.

Group: a negative control group, five test groups.

Procedure: Weaning rats of five test groups were fed with the basal diet fumigated with COS gas of corresponding doses. The rats were killed after 6, 12 and 18 months dosing respectively to examine hematological and biochemical

indicates.

Results

Six-month test

Hb of the fourth, the fifth and the sixth groups of female were lower than that of control group.

Hb of the third and the fourth groups of male were lower than that of control group.

GPT of the fourth, the fifth and the sixth groups of male were higher than that of control group.

AKP of the sixth group of male were higher than that of control group.

The results showed that 20g/m³ would be considered as the MNL of male, and 50g/m³ of female in six month test.

Table 17. Biochemical examination after 6 months dosing ($\bar{x} \pm s$).

Sex	Group	concentration of COS (g/m ³)	number of rats	Hb (g/L)	TP (g/L)	ALB (g/L)	BUN (mM/L)	GPT (U/L)	AKP (U/L)
Male	1	0	8	150.4±12.8	82.9±3.5	44.5±3.4	7.1±1.4	86.5±10.4	280±83.8
	2	20	8	148.4±9.81	80.6±2.9	40.9±3.1	7.8±1.4	99.0±14.4	273±44.7
	3	50	7	134.1±46.4*	80.0±3.7	42.5±3.4	6.2±1.5	87.7±11.0	269±59.1
	4	100	8	127.5±52.6*	84.3±5.8	41.8±3.3	7.7±1.3	101.1±15.4*	283±83.1
	5	200	8	155.0±15.0	83.6±5.2	41.5±3.2	6.7±0.8	101.3±13.0*	294±112.8
	6	500	8	153.5±50.8	79.1±3.6	40.9±2.6	6.5±0.8	102.2±14.7*	420±81.8*
Female	1	0	8	133.3±9.62	74.0±4.6	39.4±3.0	6.2±1.0	67.7±26.0	228±62.1
	2	20	8	125.9±6.69	74.1±5.1	39.4±3.4	7.0±1.2	75.1±13.2	300±113
	3	50	8	127.0±13.6	75.8±4.3	40.8±3.2	6.5±1.1	69.8±14.2	225±44.7
	4	100	4	115.0±5.86*	74.6±5.5	38.5±2.4	7.7±1.3	86.9±16.6	291±83.3
	5	200	8	105.8±7.85*	74.2±6.0	38.8±4.6	7.4±0.4	93.6±15.3	228±102
	6	500	8	104.5±15.4*	76.7±4.8	39.4±3.1	7.4±0.6	91.8±11.6	322±105

note: 1. negative control group

* compared with the negative control group $P < 0.05$

Twelve-month test

Hb of the fourth, the fifth and the sixth groups of male were lower than that of control group. No differences of other indication of test groups were observed with control

groups.

No differences were observed between female groups

The results showed that 100g/m³ would be considered as the MNL of male.

Table 18. Biochemical examination after 12 months dosing ($\bar{x} \pm s$).

Sex	Group	concentration of COS (g/m ³)	number of rats	Hb (g/L)	TP (g/L)	ALB (g/L)	BUN (mM/L)	GPT (U/L)	AKP (U/L)
male	1	0	9	161.1±7.08	78.2±3.7	32.1±1.6	6.5±1.6	147.7±9.99	236±18.7
	2	20	9	155.8±11.1	76.2±5.2	32.0±2.7	6.5±1.4	145.2±8.06	261±31.8
	3	50	10	158.9±8.31	82.5±2.6	29.2±4.7	5.6±0.5	151.4±8.49	257±65.1
	4	100	10	150.8±6.18*	78.2±1.1	31.6±1.1	5.7±1.0	150.0±7.71	170±52.3
	5	200	6	140.3±7.05*	79.4±3.7	33.4±3.8	6.7±1.0	162.3±13.1	222±77.2
	6	500	10	125.9±4.65*	85.0±3.8	42.9±3.6	6.4±1.0	159.4±43.9	241±70.0
female	1	0	11	152.8±5.18	80.5±4.2	41.2±2.4	6.8±0.4	129.1±19.5	272±72.4
	2	20	10	155.1±5.11	79.5±4.0	41.5±1.9	7.7±1.0	163.6±61.2	232±50.3
	3	50	10	149.8±8.59	79.3±4.0	42.1±2.6	6.8±0.9	163.6±36.5	343±128
	4	100	10	152.0±6.10	84.2±0.9	42.9±3.8	7.6±1.0	169.3±28.1	340±80.5
	5	200	10	144.3±5.77*	82.8±5.4	42.9±3.6	6.4±0.6	142.0±23.0	297±87.0
	6	500	10	148.6±7.532	77.9±7.9	40.5±3.6	6.4±0.6	160.7±29.1	293±100

note: 1. negative control group

* compared with the negative control group $P < 0.05$

Eighteen-month test

Hb of the third group of male was lower than that of control group. No differences of other indications of test groups were observed with control groups.

Hb of the sixth group of female was lower than that of

control group.

GPT of the fifth group and AKP of the fourth of male were higher than those of control group.

The results showed that 50g/m³ would be considered as the MNL of male, and 100g/m³ of female.

Table 19. Biochemical examination after 18 months dosing ($\bar{x} \pm s$)

sex	Group	Concentration of COS (g/m ³)	number of rats	Hb (g/L)	TP (g/L)	ALB (g/L)	BUN (mM/L)	GPT (U/L)	AKP (U/L)
male	1	0	9	150.7±9.39	67.2±3.7	34.0±3.5	5.9±0.9	74.6±6.77	196±94.9
	2	20	9	151.4±6.81	70.4±3.9	32.6±2.8	7.5±1.4	81.1±16.9	232±86.8
	3	50	10	136.7±6.11*	70.9±3.6	29.5±2.7	6.7±1.6	102.0±18.3	264±83.2
	4	100	10	147.7±6.94	69.4±3.1	33.0±2.3	6.6±1.3	114.8±66.1	318±59.4
	5	200	6	148.8±10.68	70.4±3.9	33.1±3.0	5.5±1.1	76.2±11.4	181±65.0
	6	500	10	149.4±11.34	72.8±5.8	34.1±2.7	6.3±0.9	93.4±21.8	223±65.7
female	1	20	11	141.5±10.77	80.1±5.5	35.2±5.2	6.8±1.1	65.8±14.4	126±68.1
	2	50	10	137.7±17.26	75.4±4.5	33.5±4.4	6.4±1.1	46.6±8.48	94±51.9
	3	100	10	143.4±10.77	80.9±2.7	34.7±3.2	6.8±0.7	48.9±12.8	95±43.4
	4	200	10	132.0±11.50	80.4±7.5	33.5±2.9	6.1±1.3	74.3±21.8	248±115*
	5	500	10	129.4±11.15	76.7±4.4	33.8±4.5	5.8±1.0	81.7±30.9	171±93.7
	6		10	115.9±8.37	79.8±5.1	34.3±3.3	6.6±0.8	61.2±111.0	135±51.8

note: 1 negative control group

* compared with the negative control group $P < 0.05$

Twenty-four-month test

AKP of the fifth group of male was lower than that of control group.

Hb of the fifth group of female was lower than that of control group.

No differences of other indicaty of test groups were

observed with control groups.

No special pathological injurions was observed in the test groups

The results showed that 100g/m³ would be considered as the MNL of male and female.

Table 20. Biochemical examination after 24 months dosing ($\bar{x} \pm s$).

sex	Group	Concentration Of COS (g/m ³)	number of rats	Hb (g/L)	TP (g/L)	ALB (g/L)	BUN (mM/L)	GPT (U/L)	AKP (U/L)
male	1	0	11	149.4±16.6	73.0±5.9	34.5±4.2	10±3.2	237.6±70.7	157±9.72
	2	20	7	150.4±27.4	77.8±2.7	32.2±3.4	9.8±1.9	242.7±26.1	154±7.11
	3	50	6	148.5±9.19	77.1±0.3	38.0±3.0	10±0.3	264.0±76.4	137±10.1
	4	100	6	139.0±16.51	78.9±9.8	36.5±4.0	8.5±0.5	270.3±84.8	149±25.8
	5	200	11	143.4±9.50	72.7±10	33.6±3.5	9.5±4.0	277.1±46.9	128±43*
	6	500	7	140.2±22.6	71.3±5.3	33.6±5.0	7.4±1.6	284.9±105	142±13.7
female	1	0	11	157.6±9.72	82.4±8.8	40.4±5.1	9.6±3.1	154.8±24.6	157±59.6
	2	20	10	153.5±7.11	75.1±4.4	40.2±3.6	9.9±1.2	135.0±53.4	140±77.4
	3	50	11	137.1±10.1	73.8±9.9	34.9±7.4	10±4.8	146.5±33.0	175±70.7
	4	100	8	149.4±25.8	80.5±4.5	36.5±5.0	8.5±2.7	136.5±43.9	192±74.5
	5	200	9	127.9±43.6*	76.2±7.8	37.1±5.0	10±1.5	132.7±24.4	167±68.9
	6	500	12	142.0±13.7	77.3±3.0	39.8±2.8	11±1.8	174.3±48.7	142±57.7

note: 1 negative control group

* compared with the negative control group $P < 0.05$

Carcinogenesis Test

Methods

Animal: Weaning rats were assigned to six groups.

Group: a negative control group, five test groups.

Procedure: Weaning rats of five test groups were fed with the basal diet fumigated with COS gas of corresponding doses. The rats were killed after two years' dosing to

examine the pathological and tumorous changes.

Results

Animals' lives

The average lives of the third, the fourth and the fifth groups of male were lower than that of the control group.

No differences of average lives in the test groups of female were observed with control groups

The results showed that 20g/m³ would be considered as the MNL.

Table 21. Animals' lives ($\bar{x} \pm s$).

Group	Concentration of COS (g/m ³)	number of animals	average life of male(days)	average life of female(days)
1	0	25	676.56 ± 89.73	697.44 ± 73.88
2	20	25	617.60 ± 130.3	664.12 ± 98.61
3	50	25	540.96 ± 141.1 *	627.74 ± 136.9
4	100	25	580.68 ± 137.6 *	590.60 ± 140.6
5	200	25	603.80 ± 173.6 *	647.24 ± 91.44
6	500	25	632.64 ± 105.2	647.12 ± 109.9

* compared with the negative control group $P < 0.05$

Pathological examination

There were some common pathological changes in all groups. But no difference was observed between the test groups and the control group.

Tumor of adrenal gland, breast and pituitary were

observed in some rats. Most of the tumor were sporadic, automatic and not malignant.

The results showed that COS did not cause special injury and increase automatic tumor.

Table 22. Common pathological changes ($\bar{x} \pm s$).

Group	Concentration Of COS (g/m ³)	number of rats	pneumonia pulmonary abscess	degeneration of liver cell	renal tubules abnormalities
1	0	24	2	13	11
2	20	21	3	9	11
3	50	13	2	4	5
4	100	14	2	3	9
5	200	21	3	7	14
6	500	21	0	7	6

Table 23. Number of tumor in all groups ($\bar{x} \pm s$).

Group	Concentration of COS (g/m ³)	number of rats	adrenal tumor	pituitary adenoma	adenoma of breast	fiber tumor	teste tumor	other tumor	total
1	0	24	2	0	1	1	2	0	6
2	20	21	1	2	4	0	0	2	9
3	50	13	2	0	3	1	0	2	8
4	100	14	0	1	1	1	0	1	4
5	200	21	1	0	1	1	0	2	5
6	500	21	0	2	1	1	1	0	5

Conclusion

The four stage toxic study showed:

1. COS was a kind of low toxic fumigant. It had no irritation to eye and derma. LC_{50} of COS $>2000\text{mg}/\text{m}^3$.
2. COS had no mutagenesis effect.
3. The subchronic test showed that the MNL of COS was $20\text{g}/\text{m}^3$.
4. The teratology test showed that MNL of COS was $50\text{g}/\text{m}^3$.
5. The generation test showed that MNL of COS was $50\text{g}/\text{m}^3$.
6. The chronic and carcinogenesis test showed that MNL of COS was $20\text{g}/\text{m}^3$. COS did not cause any special injury.

In conclusion, COS was a kind of low toxic and safe fumigant. Its NML was $20\text{g}/\text{m}^3$.

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