# An investigation of maximum allowable concentration of sulfolane in surface water

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**Published on Journal of West China University Medical Society 18(4), 376-380. 1987.**

**Abstract**

The authors investigated different sulfolane concentrations in surface water and also performed the field study. The results showed that the practical threshold value of sulfolane affecting the organoleptic property (order) of water amounted to 10.6 mg/L. The sulfolane concentration did not inhibit the process of biochemical oxygen demand (BOD). The chronic threshold and no-effect doses for sulfolane are 2.5 and 0.25 mg/kg bw bodyweight, respectively. There is no mutagenic effect and no teratogenic effect at 280mg/kg. Based on these results, the authors recommended a maximum allowable concentration (MAC) of 5 mg/L in drinking water for humans. Combined with the field study result, the authors considered the MAC also safe for humans.

**Introduction**

Sulfolane, C4H8SO2, colorless solid, molecular weight 120.14, boiling point 285°C, changes into clear liquid at 28°C. As an excellent industrial solvent, it also can be used as a desulfurization agent in the purification of natural gas. It is discharged together with the effluent of the desulfurization plant into the river and might cause health hazard. China currently doesn't have an established sanitary standard of sulfolane in surface water. In order to protect environment and prevent water source from pollution, it is necessary to establish a maximum allowable concentration (MAC) of sulfolane in surface water. Five experiments were carried out incuding the effect of sulfolane on the organoleptic properties (order) of water, the effect of sulfolane on the process of self-purification of water (BOD test), the general toxicity (acute, subacute, and chronic), mutagenicity and teratogenicity tests.

**Research and Experiments:**

## 1. The effect of sulfolane on the organoleptic properties (order) of water

Ten young adults with normal sense of smell were selected for the test. The smell intensities were examinated at the concentrations of 0, 0.1, 0.5, 2.5, 12.5, 62.5, 312.5mg/L sulfolane solution. The twelve experiments showed that the smell intensity increased from grade 1 to grade 5 when the concentration increased from 0.5mg/L to 312.5mg/L. According to statistic analysis of these test data, it is concluded that the lowest threshold value of sulfolane affecting the organoleptic property (order) of water amounted to 1.79mg/L and the practical threshold (actual permissible limit) was 10.6 mg/L. Therefore, in order to keep the normal water smell, the sulfolane concentration should be less than 10mg/L.

**2. The effect of sulfolane on the process of self-purification of water (BOD Test)**

The 0, 125, 250, 500, 1000, 2000, 4000mg/L sulfolane solutions were used in BOD test. All the tests were repeated seven times, with the same results as shown in Table.
The test indicated that the biochemical oxygen demand increased when the sulfolane concentration increased. When the sulfolane concentration reached as high as 4000mg/L in water, it did not inhibit the process of biochemical oxygen demand (BOD).

**3. General Toxicity (acute, subacute, and chronic) Study**

1. Acute Toxicity Test
The $LD_{50}$ were determined in the acute toxicity tests on white mice, rats and guinea pigs and found to be 2504, 2343 and 1445 mg/kg respectively. The tests showed that, after a few minutes following oral administration of the sulfolane, these three test species all became more active, short of breath, and demonstrated rigid tails, twitching, rear leg shaking, and stiffening, with some lying down and dying right away.

2. Cumulative Toxicity Test
The sulfolane were orally administered to the rats at increasing doses. The test results indicated that the cumulative index on the rats was greater than 7, and there is no significant cumulative effect.

3. 90 days Oral Administration Test
The 80 rats and 80 guinea pigs were selected for the test. They were put into the low, middle, high dose groups of 55.6, 167 and 500mg/kg and the control group. After 90 exposure-days, the animals were bled to death via femoral artery for biochemical index, organ index and pathological examinations. The test result showed that the Serum A1P activity decreased in guinea pigs in the low and middle dose groups ($P<0.05$, $P<0.01$) and their white blood cells counts also decreased ($P<0.05$). In the high dose group, in addition to the decrease of white blood cells counts ($P<0.05$), the ascorbic acid content in adrenal gland also decreased ($P<0.01$). But the rats in the low and middle dose groups didn't have any change in all the parameters. The rats in high dose group experienced higher urine volumes, their urine γ-GT activity increased ($P<0.05$), serum A1P activity decreased ($p<0.05$), ICD decreased ($P<0.05$), thrombin decreased ($p<0.01$), with no other changes observed for other parameters. It is concluded that the rats and guinea pigs got affected by the sulfolane more in their blood systems, livers and kidneys than in other organs, and the guinea pigs were more sensitive.

4. Chronic Toxicity Test

The guinea pigs which had just stopped breast-feeding were randomly selected and put into four dose groups of 0.25, 2.5, 25, and 250 mg/kg bw and the control group. Each group contained forty guinea pigs, with equal numbers of males and females. The guinea pigs were exposed to sulfolane for six months in each of the dose groups. Biochemical and pathological evaluations were conducted on a subset of each dose group following three months of exposure, the detailed is the following: the GPT is 40.8 and 45.8 u/100mL for the dose group of 25 and 250 mg/kg bw. The GOT is 71u/100mL for the dose group of 250 mg/kg bw. The marrow cell numbers is 10.99, 12.25 and $10.56x10^4/mm^3$ respectively for the dose group of 2.5, 25 and 250 mg/kg bw. The control group had the GPT 59.4u/100mL, the GOT 106u/100mL and the marrow cell number $16.43x10^4/mm^3$ respectively. The three parameters (GPT, GOT and marrow cell number) in each dose group were obviously lower than in the control group. (The F value for the parameters was 4.33, 32.2 and 4.28 respectively. P value <0.05) There is no change observed in the dose group of 0.25 mg/kg bw compared with the control group. Pathological tissue inspection indicated the main pathological change involved shrinkage of white pulp in the spleen. Compared with the control group, the pathological change rate for each group was 0/14, 1/14, 2/14, 6/14 and 0/14(the control group) respectively. Only minor Pathological changes were observed in these dose groups and failed to exhibit a dose-response relationship.

After six months of exposure, the liver biochemical index for the 250 mg/kg bw male guinea pig group was 40.2, with 33.9 for the control group. This is a big difference ($F=5.32$, $P<0.05$). The GOT increased only in the dose group of 25 mg/kg bw. Pathological examinations indicated the change rates in fatty deposits were 0/22, 2/26, 4/25, 7/22 and 0/25 for each dose group and the control group, exhibiting a dose-response relationship. The rates in the significant fatty deposits change in the liver tissue for the 2.5, 25, and 250 mg $kg^{-1}$ bw exposure groups were 1/26, 2/25 and 5/22 respectively and become increased with increasing dose. At the same time Shrinkage of spleen white pulp was also noted in these three dose groups , the rates were 2/26, 2/25 and 7/22 respectively, exhibiting a dose-response relationship. The decreasing cell counts in spinal marrow were also noted in these three dose groups. No biochemical or pathological changes were found in the 0.25 mg/kg bw dose group. No other biochemscal index and Haematology index difference were noted between the dose groups and control group. Based on these study results, the authors reported a chronic threshold and no-effect doses for sulfolane of 2.5 and 0.25 mg/kg bw bodyweight, respectively.

## 4. Mutagenicity Test

1. Ames Test

The bacterial mutagenic activity of the sulfolane was investigated in five bacterial strains $TA_{1535}$, $TA_{1537}$, $TA_{1536}$, $TA_{98}$ and $TA_{100}$. These tests were conducted either in the presence or absence of an $S_{-9}$ at the concentrations of 0, 2, 20, 200 up to 2,000 μg per plate. The compounds N-methyl-N'-nitro-N-nitrosoguanidine and 2- acetamido fluorine

were used as the positive control. It is concluded that the sulfolane was not mutagenic to the above test strains.

2. Mice Marrow Erythrocyte Micronucleus Test
The mice which were 7 weeks old were selected for the study. The sulfolane was administered orally to mice at dose levels of 1000, 500, 250, 125, 62.5 mg/kg. The cyclophosphamid was used as the positive control and the DI water as the solution control. The test followed the Schimid method except that the sulfolane were orally administrated and the bone marrow collected at breastbone. The test results indicated that there was no big difference in micronucleus counts between the dose groups and the negative control. But a big difference existed in micronucleus counts between the dose groups and the positive control. It was concluded that, under this test conditions, the sulfolane can not cause micronucleus counts to increase in mice marrow erythrocyte.

3. Sister Chromatid Exchange (SCE) Assay
The lymphocytes in human peripheral blood were lab-cultured. Mitomycin C was used as the positive control and the sterilized DI water as the negative control. Based on the QinLong method [4], the sulfolane was given to different dose groups of 0.01, 0.1, 1 and 10 mg/ml. The test result showed that the number of SCE per chromosome was 3.24±1.09, 3.60±1.19, 4.08±1.63 respectively in the first three dose groups; but in the dose group of 10mg/mL, the number of SCE per chromosome was very low because the cell growths got depressed. According to the statistical analysis, there were no significant difference observed between the negative control group (3.28±1.84) and each dose group ($P>0.05$). Therefore, under the conditions of this assay, the sulfolane was considered no significant effect on the production of SCE *in vitro* for human peripheral blood lymphocytes.

**5. Teratogenicity Test**

The Chinese kuenming mice which had just 6-15 days pregnancy were selected for the study. The sulfolane was administered orally to the mice daily at dose levels of 840, 280, and 93 mg/kg (equivalent to1/3, 1/9, 1/27 $LD_{50}$ respectively). The compound N', N-METHYLENE-BIS (2-AMINO-5-SULFHYDRYL-1,3,4-THIADIANOLE was used as the positive control and the DI water as the negative solution control. At the 18th day of pregnancy, the fetuses were taken out, and then the fetus bodies, organs and skeletons were examined. The test indicated that there were no fetus appearance, organ and skeleton abnormality in each dose group in comparison with the negative control. But the number of fetus absorption in the pregnant mice was 30.16%, which was higher than the negative control (13.53%) at the highest dose group of 840mg/kg. The skeleton abnormality were also observed in this group, including breastbone malposition, rib fuse and rib ramification. There were some differences observed between this dose group and the negative control group ($P<0.01$). But there were no abnormality observed in second highest group (280mg/kg).

**Field Study**

A high concentration of surfer was found in the nature gas produced in Sicuan Province of China. This high level surfer had to be removed before use. The sulfolane was used as a desulfurization agent in the purification of this surfer-contained natural gas in the Sicuan Zhongba and CuanDong natural gas production facilities. The trace sulfolane along with other chemicals were found in the effluent of these natural gas production facilities. In the winter of 1984, the public health department of Sicuan Province inspected the effluents from the CuanDong natural gas production facility, with the sulfolane concentration of 31mg/L found in the effluents untreated and 8mg/L treated. We toured the facility and found that the effluents contained base, waste oil and surfer-contained compounds. The discharge rate was 55 ton/hour. After the effluents went through the filtering, precipitating processes, the waste water flowed into the Pei River via a pipe. The residents down the river accessed the river water at different locations and the river water also was used to irrigate the farm lands.

**Discussion**

1. The effect of sulfolane on the process of self-purification of water (BOD Test) was listed in Table. It is concluded that the biochemical oxygen demand increased with increasing sulfolane concentration. And the sulfolane concentration wasn't found to inhibit the process of biochemical oxygen demand (BOD), which indicated that the sulfolane can only affect the consumption of oxygen in surface water. When the sulfolane concentration reached as high as 4000mg/L in water, the five day biochemical oxygen demand (BOD) was only 5.39mg/L, indicating that its oxygen consumption was very low. Therefore, the effect of sulfolane on the process of biochemical oxygen demand (BOD) was very small and no realistic meaning. In order to prevent the sulfolane from affecting the process of biochemical oxygen demand (BOD), the sulfolane concentration in the effluents should be limited according to the surface water oxygen solubility and BOD.

2. The acute toxicity tests on white mice, rats and guinea pigs indicated the sulfolane is not very toxic, which matched the reference [5]. In chronic toxicity test, starting from the dose group of 2.5 mg/kg bw, the test animals showed decreasing cell counts in marrow, main pathological change involved shrinkage of white pulp in the spleen, and significant fatty deposits change in the liver tissue. A dose-response relationship was also exhibited. It is concluded that the sulfolane can affect liver, kidney and marrow functions, with a chronic threshold dose for sulfolane of 2.5mg/kg bw bodyweight recommended.

3. Many studies indicated that a lot of chemicals can intoxicate fetuses more than their mothers, causing fetus abnormality and slow growth. It is noted in this study that, at the dose of 840mg/kg, the breastbone and rib abnormality rate and the number of fetus absorption in the pregnant mice were both higher than the negative control ($P<0.01$). There was no difference in the dead fetus rate observed between this dose group and the negative control group, indicating the sulfolane mainly caused skeleton abnormality and fetus absorption. There was no abnormality observed in the dose group of 280mg/kg. Because the sulfolane concentration in drinking water is actually very low, it is unlikely

that the sulfolane in drinking water can cause abnormality. Based on the above results, the toxicological limiting index and the field study, a MAC of salfolane of 5 mg/kg in surface water is suggested and considered practical.

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