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Genotoxicity of heated cooking oil vapors

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Summary

Epidemiological studies of lung cancer in Chinese women indicated that factors other than cigarette smoking are related to lung cancer risk. A case-control study suggested that indoor air pollution, particularly from cooking oil emissions, may be involved. Condensates of volatile emissions from rapeseed and soybean cooking oils were prepared and found to be genotoxic in short-term tests including the Salmonella mutation assay, SV50 forward-mutation assay, and sister-chromatid exchange assay, as well as the micronucleus assay in mouse bone marrow. In contrast, condensates from rapeseed oil with butylated hydroxyanisole or hydrogenated rapeseed oil were not mutagenic, implicating oxidation products as the cause for mutagenicity. Peanut oil and lard condensates were not mutagenic in any assay. The association of exposure to Chinese rapeseed cooking-oil emissions and lung-cancer risk may be related to the mutagenic component of these condensates.

The age-adjusted annual lung-cancer incidence among females in Shanghai during the 1970's and 1980's was 20 per 100000 persons, one of the highest rates in The People's Republic of China and in the world (Gao et al., 1987). These rates are surprising because only 11.8% of Shanghai women over 40 years old are tobacco smokers; so that the estimated population attributable risk of smoking for lung cancer in females is 19.3% (Gao, 1986). Furthermore, hospital records have indicated that most of the lung tumors are adenocarcinomas (61%) (Gao et al., 1987), which are less strongly related to tobacco use (Gao, 1986). A retrospective analysis of 452 lung cancer patients in San Francisco indicated that 39.3% of lung cancers are adenocarcinomas in Chinese women compared to 17.4% in non-Chinese women (Green and Brophy, 1982). Thus, the etiology for these cancers may be an interaction of some traditional and nontraditional exposures.

A case-control study in Shanghai of 672 female lung-cancer patients and 735 population-based

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controls identified an association to wok cooking, especially when rapeseed cooking oils were used (Gao et al., 1987). The risk of lung cancer was independently related to the subjective variables of eye irritation and house smokiness during cooking. The highest relative risk (RR) was in the categories of persons using rapeseed oil and most frequently reporting eye irritation (RR = 2.8, 95% CI = 1.8–4.3). While Shanghai is known to have a significant amount of outdoor-air pollution, this association of cooking-oil vapors is independent, as evidenced by both cases and controls residing within Shanghai. In addition, the **RR** increased with the number of different dishes per week prepared by stir frying, deep frying or boiling (Gao et al., 1987).

Recently, another lung-cancer case-control study of 965 female patients and 959 controls in Shenyang and Harbin (urban cities in north-east China) indicated that lung-cancer risk also was related to indoor-air pollution, but from coal heating, kang (brick beds heated by coal), and cooking stoves (Wu-Williams et al., 1990). This study also found that lung-cancer cases more often reported smoke from cooking, eye irritation, and had an increased number of meals cooked by deep frying. In these cities, soybean oil rather than rapeseed oil is predominantly used. In a multivariate, unconditional logistic regression analysis, in addition to tobacco smoking, deep-frying and eye irritation ranked first and second, respectively, to have significant associations with lung cancer (Wu-Williams et al., 1990). Coal use, however, cannot be a factor in Shanghai because gas is the most common type of heating and cooking fuel.

Soybean and unrefined rapeseed oils **(URSO)** are used most often for cooking in Shanghai, with over 95% of women reporting the use of both products. Refined and unrefined rapeseed oil are both available in China, but the former is costly and rarely used. Rapeseed oil is produced by steaming (140°C) at pressing. The procedure for refinement in processing facilities includes the elimination of free fatty acids by neutralization and extraction with water, filtering with charcoal to eliminate color and heating the oil to 180°C under vacuum to eliminate odors. Because URSO emits an obnoxious odor during cooking, the oils are typically pre-heated in the home to high temperatures (270–280°C) for several minutes, thereby reducing the odor. This practice however, increases exposure to URSO vapors. Searching for corroborating evidence to the epidemiological associations, we assayed condensates of volatile emissions from heated cooking oils in several short-term mutagenesis assays.

Material and methods

Chemicals. The cooking oils were purchased from common markets in Shanghai. Butylated hydroxyanisole (BHA) was obtained from the Shanghai Fourth Ye Min Food Corporation. Dimethyl sulfoxide (DMSO) was imported from Sigma Corp. (St. Louis, MO). Glass filters No. 49 are the products of Shanghai Huon Guan Paper Corp. Rat-liver homogenates (S9) were prepared from male Wistar rats treated with Aroclor 1254 (Shanghai Cancer Institute). Other agents were analysis-grade. For the analysis performed in the United States (Rockville, MD), **DMSO** was purchased from Sigma (St. Louis, MO), and S9 was purchased from Microbiological Associates (Rockville, MD).

Preparation of condensates. Cooking oils were heated in a wok at 270°C (usual cooking temperatures) with an electric heating mantle. The wok was covered except for three holes — two collection filters (two filters per holder) connected to a vacuum pump (flow rate: 30 l/min for 20 min) and a thermometer. Experiments were performed outdoors in an area with the lowest possible air pollution for Shanghai. **A** separate analysis on-site to observe background mutagenicity was performed by operating the vacuum pump attached to the filters, with and without the oil on the filters, but without the process of heating oils in the wok. The condensates were extracted from the filters with acetone (10 ml of acetone per **4** filters) and passed through filter paper (Xin Hua 102). The acetone extract was then evaporated at 60°C under nitrogen. The mass of the condensates were determined by pre-weighing glass vials and then determining the difference in weight after acetone evaporation. Condensates in the **U.S.** were prepared exactly as in Shanghai except

that the procedure was performed in a laboratory fumehood. The condensates were too viscous to handle directly so they were diluted with **DMSO** (1 ml).

Salmonella mutation assay. The condensates dissolved in DMSO were diluted and tested with ***Salmonella typhimurium*** histidine auxotroph strains **TA98** and TAlOO (obtained from B.N. Ames, Berkeley, CA) with preincubation according to the method of Maron and Ames **(1983). 3** parallel plates were used for each dose. Briefly, test condensates **(0.1** ml), tester strain **(0.1** ml) and **S9** mix (0.5 ml) were added to sterile **13 × 100** mm capped culture tubes. The tubes were gently vortexed and incubated at **37°C** for 20 min (gentle shaking). Following this preincubation, selective top agar (2.0 ml) was added to each tube and the mixture was vortexed and overlaid onto the surface of minimal bottom agar (25 **cl).** The plates were incubated for **48–72** h. Colonies were counted by an automated colony counter. The mean number of revertants from **3** plates was designated positive if a 2-fold increase **in** revertant numbers occurred. In the U.S., the test system was identical to the above. Tester strain **TA98** was also obtained from Dr. Bruce Ames.

SV50 forward-mutation assay. SV50 strain bacteria was obtained from **Dr.** T.M. Ong (West Virginia). The test was modified from Whong (Whong et al., **1981).** Briefly, **SV50** cell culture **(0.1** ml; **1.5 x 10^6** cells), URSO condensates (0–5 mg/0.1 ml **DMSO),** L-arabinose **(20%; 0.3** ml), glucose (0.1 mg), and **S9** liver extract **(0.5** ml) were mixed and preincubated for 30 min. The mixture was then added and poured onto a **M9** bottom agar **(25** ml/plate). The plates were incubated at 37°C for 3 days. Arabinose resistant colonies were counted by an automated colony counter.

Sister-chromatid exchange assay. V79 Chinese hamster cells **(10000/cm^2)** were inoculated in 60-mm diameter plates containing minimum essential medium (MEM) with neonatal calf serum (10%), penicillin (100 IU/ml), streptomycin (100 **μg),** and **S9** liver extracts **(5%).** After incubation (37°C for **24** h), the medium was replaced with MEM containing URSO. Cyclophosphamide was used as a positive control (4 μg/ml). After exposure to URSO, the cultures were again washed. Complete MEM with bromodeoxyuridine (10 mM) was added and the cultures were again incubated **(37°C** for **24–30** h). Colchicine **(0.5** μg/ml) was added to the medium **4** h before the cells were harvested. The harvested cells were treated for **7** min with potassium chloride (0.075 **M),** fixed with methanol and acetic acid **(3/1;** v/v), and transferred to the slides. They were mounted in sodium phosphate buffer (pH 6.6), exposed to UV light **(25 min),** and immersed in 55°C of **2 x** standard saline citrate for 2 h, and stained with Giemsa **(3%).** The number of SCE in **30** metaphases were scored for each dose.

Micronuclei assay. Male Kunming mice (Shanghai Cancer Institute) were intraperi-

TABLE 1

TA98 SALMONELLA MUTATION **ASSAY.** SHANGHAI CANCER INSTITUTE

Dose (mg/plate)	Revertants/plate [a]				
	Unrefined rapeseed	Refined rapeseed	Soybean	Peanut	Hydrogenated rapeseed B
Vehicle [b]	27+ 3	37± 3	39+ 2	25±3	33±3
0.1	28+ 5	54± 6	42+ 2	22±2	33±5
0.5	48±13	106± 6	110±14	26±2	37±3
1	94+ 8	162±10	133± 9	-	28±3
2	146±10	127± 7	158±17	27±5	-
5	152±11	108±11	137±12	16±5	48±6

[a] **Positive control.** 2-acetylaminofluorene (10 μg/plate) Revertants/plate 796–1237

[b] **Vehicle.** acetone (0 1 ml/plate).

TABLE 2

TA98 SALMONELLA MUTATION ASSAY, U.S. NATIONAL CANCER INSTITUTE

Dose (mg/plate)	Revertants/plate		
	Unrefined rapeseed	Soybean	Peanut
Vehicle [a]	19 ± 1	23k 6	28 ± 5
0.1	42 ± 9	23k 9	21 ± 5
0.33	111 ± 16	45 ± 7	26f 2
1.0	157 ± 10	68 ± 8	25+ 3
3.33	29 ± 8	15k 3	15f 3
10.0	44 ± 2	9 ± 4	19 ± 3
Positive [b]	136 ± 11	181 ± 16	147 ± 16

[a] Vehicle. DMSO (0.1 ml/plate).
[b] Positive control, 2-aminoanthracene (0.5 mg/plate).

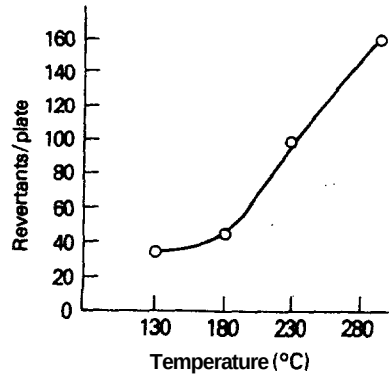

Fig. 1. The effect of temperature on condensate (0.5 mg/plate) mutagenicity measured in the Salmonella mutation assay (TA98 plus S9).

toneally injected with condensate (800 mg/kg), which was repeated in 72 h at varying doses (800–3200 mg/kg). Animals were sacrificed at varying intervals. up to 6 days later. Polychromatophilic erythrocytes from femur bone marrow were examined for micronuclei as described previously (Schmid, 1975).

Results

Salmonella mutation assay. Condensates from 2 separate batches of Chinese cooking oils including URSO, refined rapeseed oil, soybean oil, peanut oil, and lard were tested with *Salmonella typhimurium* strains TA98 with Aroclor 1254 induced rat-liver S9 in Shanghai (Table 1). Condensates from URSO, refined rapeseed oils and soybean oils were positive. This finding was confirmed in the U.S. (Table 2). The condensate from URSO refined rapeseed and soybean were mutagenic in Shanghai and the U.S. The mutagenicity of URSO condensate was dependent on the cooking-oil temperature (Fig. 1). All of the condensates were negative in TA98 without S9 and TA100 with or without S9 (data not shown). Unheated oils were all negative in TA98 and TA100 with or without S9.

We hypothesized that oxidized products of unsaturated fatty acids might be related to the mutagenicity. Different amounts of BHA were added to the URSO before cooking and condensates were collected. The results are shown in Fig. 2. BHA inhibited the mutagenicity with higher concentrations causing the greatest reduction in revertants, but not until BHA concentration reached 0.1%. The current permissible concentration by the Food and Drug Administration (USA) is 0.02%.

Hydrogenation of rapeseed-oil samples, which saturates fatty acids, was performed by the Shanghai Cereal Science Research Institute. The proportion of fatty acids for rapeseed oil and two

TABLE 3

PROPORTION OF FATTY ACIDS IN RAPESEED OILS

Oil	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Arachidonic c20:1	Erucic c22:1
Rapeseed	0.95 [a]	14.8	12.2	10.3	0.76	49.8
Hydrogenated Rapeseed A	1.3	29.7	6.8	1.0	11.5	45.0
Hydrogenated Rapeseed B	1.8	36.1	n.d. [b]	n.d.	12.0	47.0

[a] Percentage of fatty acids.
[b] Not detected.

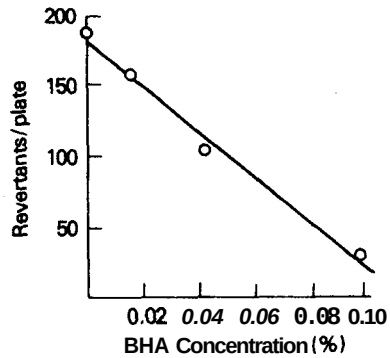

Fig. 2. Effect of BHA on URSO condensate (0.5 mg/plate) mutagenicity in the Salmonella mutation assay (TA98 plus S9). BHA was added *to* URSO at increasing concentrations. Condensate dose = 0.5 mg/plate.

hydrogenated rapeseed oils are shown in Table 3. Sample **A** contained 6.8% linoleic acid and 1% linolenic acid, sample B contained no linoleic acid or linolenic acid 3–27%. Neither hydrogenated rapeseed oil was mutagenic. Table 1 shows the mutagenicity data for the Salmonella mutation assay of sample B.

SV50 forward mutation assay. Mutagenicity data are summarized in Table 4. Aflatoxin B2 was used as a positive control. Condensates from URSO were mutagenic as evidenced by an increased number of colonies at all concentrations (0.5–5 mg/plate).

TABLE 4

SV50 FORWARD-MUTATION ASSAY

Test substance	Dose (mg/plate)	Mutants/plate
Rapeseed oil	0.5	483 ± 20
	1	644 ± 17
	2	568 ± 33
	5	475 ± 39
Aflatoxin B2	1.5 μg/plate	360 ± 13
Dimethyl sulfoxide	0.1 ml/plate	64 ± 7

Sister-chromatid exchange assay. When **V79** cells were exposed to condensates of URSO for 12 h, SCEs were increased at concentrations between 0.16–50 μg/ml for 12-h exposure and 0.8–20 μg/ml for 24-h exposure (Table **5**). When the cells were treated with condensates from URSO with 0.02% of BHA, the number of SCEs were reduced ($p < 0.05$). SCEs for condensates from hydrogenated rapeseed oil sample **A** was higher than that of the solvent control but less than the number induced by URSO condensates (4 μg/ml vs. 0.16 μg/ml for maximal number of SCEs, respectively). Condensates from hydrogenated rapeseed oil B without linoleic and linolenic acids did not increase SCE. Condensates prepared from refined Chinese rapeseed oil also were not mutagenic.

TABLE 5

SISTER-CHROMATID EXCHANGE ASSAY

Oil	Concentration (mg/ml)			
	0	0.8	4.0	20.0
Unrefined [a]	0.553 ± 0.2	0.708 ± 0.32	0.637 f0.24	0.844 f 0.27
Refined [a]	0.374 ± 0.08	0.428 ± 0.17	0.413f0.17	0.389 ± 0.21
Unrefined + BHA [a,b]	0.570 ± 0.23	0.614 ± 0.25	0.607 ± 0.29	0.612 ± 0.26
Hydrogenated				
Sample A	0.414 ± 0.02	0.432 ± 0.03	0.456 ± 0.12	0.434 ± 0.01
Sample B	0.349 ± 0.07	0.295 ± 0.04	0.302 ± 0.06	0.349 ± 0.07

[a] Difference between unrefined rapeseed oil with and without BHA, $p < 0.05$. No statistical difference between refined and unrefined plus BHA rapeseed oils.

[b] BHA, butylated hydroxyanisole at 0.02%.

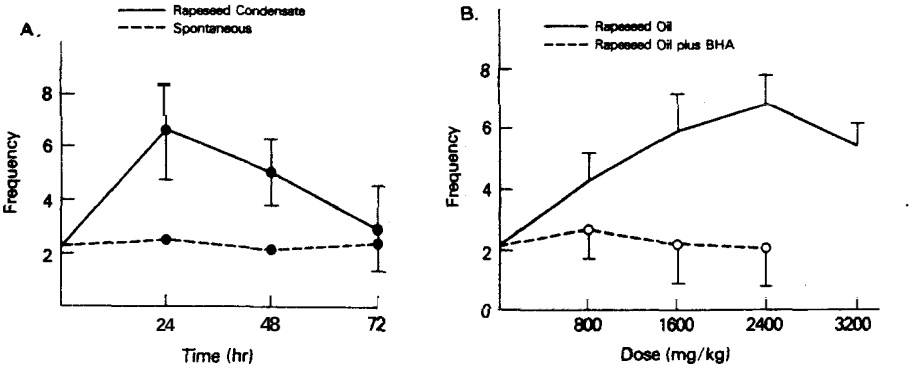

Fig. 3. Micronuclei induced by URSO condensates as a function of time (A). A dose–response curve is shown (B). Error bars represent the mean ± two standard deviations. BHA concentration in the oil is 0.02%.

Micronuclei assay. The frequency of polychromatophilic erythrocytes with micronuclei was highest at 24 h after 2800 mg/kg of URSO condensate (Fig. 3A) and a dose–response relationship was observed (Fig. 3B). Condensates from URSO with BHA (0.02%) inhibited micronuclei formation (Fig. 3B).

Discussion

Epidemiological evidence from Shanghai implicated exposure to volatile emissions of rapeseed oil as a risk factor for lung cancer (Gao et al., 1987). In Shenyeng and Harbin, cooking-oil emissions were also implicated but soybean rather than rapeseed was associated with lung cancer (Wu-Williams et al., 1990). In this study, the condensates prepared from the volatile emissions of cooking oils were tested for mutagenicity with short-term tests. URSO condensates were evidently genotoxic in the Salmonella TA98 mutation test (corrobated in two laboratories) and the SV50 forward-mutation system, both of which represent point mutations. It was also found that URSO condensates induced SCE of V79 cells and micronuclei in mouse bone-marrow cells. In all the in vitro studies, metabolic activation by rat-liver S9 homogenates was required. Thus, the positive short-term tests consisted of in vivo and in vitro treated prokaryote and mammalian cells, with 3 different biological end points. Bartsch pointed out that an agent or complex mixture with unknown carcinogenic potential showing sufficient in vitro and in vivo genotoxicity likely represents a hazard to humans (Bartsch and Malaveille, 1990), thus corroborating the epidemiological data. In these studies, soybean-oil emissions were also mutagenic in the Salmonella mutation assay but it was not tested in other assays.

Rapeseed and soybean oil contain linolenic acid which has 3 double bonds and is easily oxidized at high temperature to produce pyrolysates including aldehydes, ketones, alkanes, alcohol and alkenes (National Research Council, 1988). This study suggests that oxidized products of URSO components is mutagenic because the genotoxicity disappeared (or decreased) with addition of BHA or hydrogenation. Refined rapeseed oil also is mutagenic although it would have been predicted that the processing step of heating (180°C) to eliminate odors would decrease the amount of linolenic acid (Mounts, 1979). Fig. 1 suggests that heating to 180°C may not be sufficient for these oils.

Other studies have suggested a mutagenic potential of volatile emissions from foods and cooking oils. Alkaline extracts of air collected during beef frying increased the number of Salmonella strain TA1538 revertants with S9 (Felton et al., 1981). Samples collected in cooking areas and dining rooms of 4 restaurants also were mutagenic in TA98 or TAlOO but without S9 (Teschke et al., 1989). The differences from our results regarding TAlOO and also the requirement for metabolic activation may be due to the nature of

emissions or differences in sample collection and extraction. In another study, volatile emissions from soybean oils collected in a cold trap increased mouse bone marrow micronuclei (Liu et al., 1991), which was consistent with our results.

Although short-term studies have provided evidence that rapeseed condensates are mutagenic, further study in laboratory animals is needed to explore a carcinogenic relationship. These condensates are complex mixtures that also will require fractionation to isolate and identify putative mutagenic compounds. Further epidemiologic studies also are warranted. However, in view of the fact that condensate mutagenicity increases with the temperature of the oils, a recommendation for reducing cooking temperatures might be a strategy for preventing the exposures now occurring in some Chinese homes.

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