The Rapid Collection and Application of Cigarette Smoke Condensate

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SUMMARY

A practical system for the collection and application of cigarette smoke is described. The apparatus is judged to be easier to operate and maintain than systems employing cold traps or solvent liquid traps. Condensation of the smoke is accomplished through the use of a restricting nozzle. Application of the condensate may be an indirect or direct operation. In the indirect mode of operation, a weighing bottle is used to collect the condensate which may then be used for analytic or biologic test purposes; in the direct mode of operation, the smoke is condensed on the shaved skin of a mouse. Thus, the system permits the use of condensates which have existed only seconds or minutes in contrast to the well-aged condensates used by most investigators. The freshness of the condensates used and the conditions under which they are obtained may be important with respect to the conditions which prevail in human smoking. Animal tests are in progress which employ the techniques described herein.

INTRODUCTION

In order to provide large quantities of cigarette condensate for animal experimentation, investigators have often employed the following basic system: (a) the use of manifolds for the simultaneous smoking of numerous cigarettes; (b) cold traps in series to condense the mainstream smoke; (c) vacuum source to provide the pressure differential for puffing; and (d) control valves actuated by a timing device (2, 4, 6, 10, 15). Modifications of this basic system have been reported. For example, Gellhorn (5) did not use a manifold, and the particulate phase was trapped by electrostatic precipitation (1). In their comprehensive review of the experimental aspects of tobacco cancer research, Wynder and Hoffman (16) mentioned that the manifold system has been criticized because it puffs some cigarettes more strongly than others, and objections to electrostatic precipitation are made on the basis that it may increase the formation of organic radicals and polynuclear aromatic hydrocarbons.

Additional objections would appear to be in order relative to the recovery, processing, and application of trapped smoke condensate. In the references cited, the condensate is always dissolved in an organic solvent which must then be removed or reduced in volume by heat, reduced pressure, prolonged distillation, etc. Then the processed material may be stored for as long as two weeks to a month (4, 15), or perhaps longer, before it is used. Application to the skin is made by brush painting a solvent solution, a technic which has been recognized as giving less accurate dosage than desirable (14). While these processes may have little or no effect on the carcinogenic potency of the final preparations employed, the history of the processed material at the time of application is far different from the smoke inhaled in human smoking.

An experimental program which includes the application of cigarette smoke condensate to mouse skin is being conducted in this laboratory. Some of the objectives of this program are: (a) To reduce to a minimum the age of the condensates applied and to increase the rapidity and reproducibility with which they are applied. (b) To retain in the condensate, until applied, the chemical character of the particulate phase as it is inhaled in human smoking. (c) To devise a trapping system which would avoid the conventional cold traps and the attendant inconveniences or problems (e.g., resupplying refrigerant and avoiding plugging of the smoke pathway) and yet supply yields of condensate comparable to that experienced with cold trapping. (d) To devise a system which would permit the use of experimental filters.

The purpose of this report is to describe the apparatus and techniques employed in meeting the aforementioned objectives. The central feature of the apparatus is a restricting nozzle which impinges the smoke stream against a solid surface. In this respect, it is similar to the machines which have been reported by Neurath and Kroger (9), Seehofer and Hansen (11–13), and Mathewson (7), one or more of whom have discussed the theories involved and projected various applications. In comparison to previously described machines, it is believed that the apparatus described herein is less complicated, less costly to construct, repair, or replace, and is more suitable for the direct application of condensate to a relatively large number of test animals.

Recently, Neurath (8) has pointed out that cold trapping and solvent liquid trapping of cigarette smoke produce abnormally large quantities of N-nitrosamines as a result of prolonged contact between the precursors (secondary amines and oxides of nitrogen). He further suggests that a reasonable duplication of the N-nitrosamine production occurring in humans in the course of smoking will be obtained only when...
trapping is performed at near human body temperature and after the smoke has existed in the vapor phase for a time approximating human puff time (5 sec). Although Neurath’s discussion applies only to N-nitrosamines, the production of other toxic artifacts may occur during cold trapping and solvent liquid trapping. The technics given in this article very nearly meet Neurath’s requirements.

**APPARATUS AND PROCEDURES**

The smoking apparatus is shown in Chart 1. The round head design insures that each cigarette is subjected to an equal pressure differential between the surrounding atmosphere and the interior of the smoking head during puffing. All of the smoke condensates used are obtained by impinging the smoke stream at high velocity against a glass surface at room temperature or directly against the shaved skin on the back of a mouse. Under optimum conditions, the visible portion of the smoke stream is essentially completely precipitated by this technic. When the condensate is to be collected prior to application to a mouse, the lower end of the device is sealed off with a standard weighing bottle which receives the condensate. When the condensate is to be delivered directly to a mouse, the weighing bottle is replaced with the modified ground glass joint. The mouse is held by the tail, placed on a 6 x 6-inch piece of wire gauze, and positioned so that the shaved area of the back forms a seal at the 1-cm opening at the bottom of the joint.

The restricting nozzle tip is adjusted to a height of approximately ¾ inch above the surface receiving the condensate by slipping the inner 8-mm piece of tubing up or down through the stoppers at the time the apparatus is assembled for use. Height adjustment affects condensation efficiency and spattering of the condensate. The diameter of the orifice in the restricting nozzle is critical. It should be of such diameter that good precipitation occurs and should offer resistance to gas flow greater than the total resistance to gas flow through the 15 cigarettes to be smoked. When this latter condition is met, the composite puff volume remains essentially the same whether the puff being taken is the first or last on the burning cigarettes because the gradually decreasing resistance to flow through the burning cigarettes is negligible compared to the flow resistance through the orifice. The orifice being used is 0.070 inch in diameter and one inch long.

When filtered smoke is desired, filter cigarettes may be used or reusable cigarette smoke filters may be attached to the side arms to hold the cigarettes. Chart 2 shows a modified commercially available filter. Filters are attached to side arms with sections of rubber tubing. The cellulose filter plug may be treated with water, chemical solutions, or replaced by a filtering medium other than cellulose. Modification of the filter was necessary to facilitate attachment to the smoking arms, to permit easy disassembly for renewal of the filter, and to prevent the passage of smoke around the filter plug—an event which was observed to occur occasionally in the unmodified filter. The washer shown in Chart 2 facilitates the removal of the metal cigarette holder during filter disassembly. The Teflon piece is essential in preventing the passage of smoke around the filter plug. When assembled as indicated, the Teflon piece provides pressure (by shortening the distance allotted to the filter plug as normally manufactured) against the cellulose filter plug and causes it to distend against the sides of the encasing plastic tube. Cigarettes are placed in the metal cigarette holder which is a part of the filter as purchased. These same holders are a convenient means of holding cigarettes when nonfiltered smoke is to be used. In this latter application, the metal holders are separated from the filters and attached to the side arms with short sections of rubber tubing.

Mice which are treated with the unaltered condensates collected in weighing bottles receive their doses by means of the plastic applicator shown in Chart 3. This device is cast from Silastic RTV 501 (Dow-Corning) with a depression in one side of the piece. The depression is sized at the time of casting (or drilled out after setting of the plastic has occurred) to hold the specified dose of condensate. In use, the plastic piece is held in one hand, depression side up, with the thumb and fingers at natural grasping points at the edge. The depression is filled with the aid of a small spatula. Then the piece is flexed by squeezing the thumb and fingers together, inverted, and wiped from tail toward head along the mouse’s back. The delivery of condensate is both rapid and positive.

The vacuum source for the smoking setup is provided by a
John D. Millar, John W. Rhoades, and Donald E. Johnson

that is, on the average, between 20-25 mm, 25-30 mm, or chosen, the number of puffs required to produce a butt length to smoke to an exact butt length. A practical solution to this smoking conditions or at other conditions if desired. When meter attached to the inner 8-mm glass tube shown in Chart 1.

Welch vacuum pump, Model 1405. The pump is connected to the control system of a Phipps & Bird smoke sampling apparatus, which is used to regulate puff duration and puff interval. The desired puff volume is obtained by adjusting a needle valve air bleed control placed in the system between the pump and the Phipps & Bird machine. Daily, before smoking operations begin, the composite puff volume is checked and reset, if necessary, on the basis of readings obtained with a gas wet-test flow meter attached to the inner 8-mm glass tube shown in Chart 1.

The apparatus controls may be adjusted to operate at standard smoking conditions or at other conditions if desired. When smoking a number of cigarettes at one time, it is not practical to smoke to an exact butt length. A practical solution to this problem is to determine, after other conditions have been chosen, the number of puffs required to produce a butt length that is, on the average, between 20-25 mm, 25-30 mm, or within some other desired limits. Once the required number of puffs has been established, the glass smoking head is then fabricated with this same number of side arms.

In the illustration which follows, each cigarette is puffed 15 times after lightup. The amount of condensate delivered during the first puff is less than that from the second puff, and so forth, with the last puff contributing a substantially greater quantity than the first puff. Each mouse receiving the direct impingement treatment receives one composite puff (15 individual puffs) per treatment. Obviously, then, if a mouse were to receive a dose consisting of a composite of 15 initial individual puffs, its dose would be far less than that received by a mouse receiving a dose consisting of a composite of 15 final individual puffs. In order to avoid the administration of unequal doses, the following scheme is employed to deliver repetitively a composite puff which theoretically delivers a constant amount of condensate which is equal to the condensate from an average cigarette. At startup, only Cigarette No. 1 is lit during the first puff, then Cigarette No. 2 is lit during the second puff, etc., until all 15 cigarettes have been lit. During the interval between puffs 15 and 16, Cigarette No. 1 is replaced by Cigarette No. 16 which has just been lit on an adjacent smoking head. Therefore, the 16th puff after startup is a composite of 15 puffs from 15 cigarettes, one of which is receiving its first puff, another its second puff, and so on down the line to the one cigarette receiving its 15th and final puff. Impingement begins with the 16th puff following startup. Before the 17th puff occurs, Cigarette No. 2 is replaced by Cigarette No. 17, lit on the adjacent machine, and thus the 17th puff delivers a composite puff from 15 cigarettes having the same history of smoking as those 15 cigarettes producing the immediately preceding composite puff. The process of replacing the cigarette which has just finished its 15th puff with a freshly lit cigarette is continued until the required number of composite puffs has been delivered. Thus, to treat 50 mice with one composite puff each, 65 cigarettes are expended, including the 15 used in the startup procedure. When condensate is collected in weighing bottles, cigarettes may be lit and smoked in batches of 15 rather than in the sequential method just described.

RESULTS AND DISCUSSION

The apparatus described has been in use about six months and no significant operational problems or failures have been encountered. The simplicity and rapidity of the procedure permits the treatment of a large number of mice in a short period of time.

Maintenance is simple. No special trap has been in use to protect the vacuum pump. A weekly change of the oil has kept the pump performing satisfactorily. The glass parts and the restricting nozzle are cleaned daily with a 50:50 mixture of benzene:methyl alcohol. Because the smoke stream passes through the restricting nozzle at high velocity, the nozzle is essentially self-cleaning and does not plug up unless a small piece of tobacco is sucked into the aperture. This has occurred rarely.

The pressure at the side arm to the vacuum source has been measured at about 0.8 atmosphere during a puff. This 20% reduction in pressure from atmospheric does not appear to damage the skin of either the test or control animals. The temperature at the point of condensation of the smoke stream is not known at this time, but, when the seal at the bottom of the glass joint is made with the finger or back of the arm, the condensing stream feels slightly cooler than body temperature. Neither the impinging action nor the reduced pressure produces discomfort, but only a mild tickling sensation.

The reproducibility of the condensate yields which can be expected in day-to-day operations is given by the data in Table 1. The mean of the data is 45.3 mg and the standard deviation

Chart 2. Modified commercially available filter.

The 95% confidence interval for the mean is 45.3 ± 0.5. The 90% confidence interval for the mean is 45.3 ± 0.5. The statistics are based on the assumption that sampling is from a normally distributed population. To obtain the yields shown in Table 1, 85-mm, nonfilter American cigarettes were smoked under the following conditions: Number of cigarettes being smoked at one time, 15; average puff volume yields shown in Table 1, 85-mm, nonfilter American cigarettes as a puff is being taken. The time elapsed for the smoke to travel from the point of combustion to the point of collection is estimated to be comparable to that time required for the smoke to travel from the burning point in a cigarette to the lung area in human smoking. The temperature at these corresponding areas of contact are very nearly the same. There may be some similarity in the nature of condensation action for, in both cases, the smoke travels rapidly through a narrow passage to reach an obstructing surface.

In the direct impinging procedure, the gaseous fraction as well as the particulate matter contacts the back of a mouse as a puff is being taken. The time elapsed for the smoke to travel from the point of combustion to the point of collection is estimated to be comparable to that time required for the smoke to travel from the burning point in a cigarette to the lung area in human smoking. The temperature at these corresponding areas of contact are very nearly the same. There may be some similarity in the nature of condensation action for, in both cases, the smoke travels rapidly through a narrow passage to reach an obstructing surface.

During the indirect operation, the condensate from 45 cigarettes can be collected easily in a single weighing bottle. A replacement weighing bottle can be put into position in a matter of seconds without interrupting the operation.

The rate of smoke collection and application varies with the smoking conditions chosen. Using the conditions given above, 50 mice may be treated in one hour by the indirect operation. When the direct operation procedure is employed under the conditions given and when the required dose can be supplied by a single composite puff, approximately 120 mice may be treated within one hour. Under standard smoking conditions, the rate of treatment will be one half the above rates, or less, depending upon dose requirements.

The significance of being able to treat large numbers of mice rapidly with freshly prepared material has been emphasized recently by Day (3). Furthermore, his results suggest that tumorigenic activity is higher for 24-hour condensate than for similar material stored 4 to 8 weeks.

Using the methods described herein, the treatment of large numbers of mice is feasible, and the comparison of condensates of varying ages is possible through the options of direct impingement and collection in a weighing bottle. Results of the biologic tests being conducted will be published at a later date.

REFERENCES

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