FINAL PROGRESS REPORT

Title:	**A Laboratory Model of Sick Building Syndrome**
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Acknowledgement:	**Assistance has been provided by William McArthur, PhD, consultant and Stephan Loh, technician**

Our goal was to develop a laboratory model of Sick Building Syndrome. Among the original specific aims were the following:

1: To measure the fractional retention of volatile organic compounds (VOC) by the respiratory system during brief periods of exposure, plus the measurement of their excretion in expired air following two hours of continuous exposure.

2: To test whether the rate of sighing can be used to assess symptoms and breathlessness and/or difficulty breathing.

3: To develop preliminary data on the possible relationships among the following: ambient concentration of the irritant(s), conjunctival symptoms, and changes in tear fluid composition.

4: To test the role of olfaction in modulating SBS.

5: To explore the addition to our model of (a) a cognitive testing battery and (b) a method of assessing glucose metabolism in specific brain regions, including the neocortex, basal ganglia, and cerebellum.

6: To test allergic and atopic individuals, with particular reference to assessing changes in nasal lavage fluid associated with symptoms of nasal irritation.

We made progress on specific aims 1, 3, 5a, and 5b as follows:

1: Preliminary studies of fractional uptake and excretion of VOC. The results of the preliminary studies on the fractional uptake and excretion of toluene and n-hexane were described in the interim report (date: April 17, 1992) and are in the Appendix.

3: Developing and testing techniques for correlating the effects of VOC on the composition of tear fluid and symptoms of eye irritation.

5a: Testing the effects of VOC and learning on the performance of neurobehavioral tasks.

5b: Establishing the test-retest reliability of cerebral glucose metabolism measurements.

Rationales for the objectives and reasons for research directions are described below:

3. **Eye irritation exposure:**

Rationale: Symptoms of eye irritation are often reported after SBS. Our objective was to develop an assay for changes in tear composition for possible correlation with subjective complaints.

Procedures: We developed and discarded a lavage method of collecting tear fluid which involved depositing a known number and weight of droplets of filtered, deionized water onto the eye, having the subject mix the deionized water with tear fluid by closing the eyelids and rotating the eyeball for a specified time, and collecting the excess fluid outside the eye with filter paper held against the skin. Although this procedure was minimally invasive, samples collected in this way produced negligible amounts of the assays of interest and was abandoned for that reason.

The collection method we finally adopted was as follows: A spear-shaped sponge (Weck Cel sponge) was lightly applied at varying points within the conjunctival sac at the lower eyelid for 30 seconds to absorb tear fluid. Routinely, we attempted to collect tear fluid from each eye. Although tolerated by all subjects, the procedure was slightly irritating and produced reflexive tearing in most subjects.

The system used to expose the eyes to an irritant is shown schematically in Figure 1. The undiluted test gas (1 ppm acrolein) was obtained from a commercial tank. Clean air came from a tank of commercially purified air containing less than 100 ppb of hydrocarbon contaminants. Flow from the tank of clean air was controlled by a pressure regulator and mixed with acrolein from the commercial tank to produce the desired concentration at the sampling site proximal to the goggles. During this phase, the excess gas mixture was vented to a vacuum. To being eye exposure, the gas was directed through the goggles. Flow rate through the goggles was 1.5 $L\text{-}min^{-1}$. The dead space of the goggles when attached to a subject was approximately 250 ml. The goggles were under slightly positive pressure to reduce the likelihood that the respiratory system was exposed; in the event of a leak, a low speed fan was directed at the breathing zone.

The detection limit of the gas chromatograph for acrolein, as reported by the manufacturer, was 300 ppb. Our own studies showed that the photovac did not give consistent readings below 300 ppb. We, therefore, developed an equation to predict the concentration from flow rate, based on analyses of acrolein made at varying flow rates and concentrations above 300 ppb, including factors compensating for adsorption onto the tubing and for disproportionate loss of acrolein at lower concentration levels:

$$PC_{acr} = C_{acr} * FR_{acr} * 0.92 (FR_{acr} * FR_{air}) - 0.26 * FR_{air}$$

where PC_{acr} = Predicted concentration of acrolein; C_{acr} = Acrolein concentration (1000 ppb);

FR_{acr} = Flow rate for acrolein (500 ml/min); FR_{air} = Flow rate for air.

We found virtually 100% of the desired concentration of acrolein was achieved in the goggles within 1 min. with our eye exposure system, at a flow of 1.5 $L\text{-}min^{-1}$.

We assayed for the following markers in tear fluid:

1. Lactic dehydrogenase, an enzyme involved in glycolysis, indicative of aerobic cell metabolism; an increased level in tear fluid would suggest cell damage (cytotoxicity).

2. Total proteins, which may comprise glandular secretory proteins released by irritated or damaged cells, including enzymes such as lysozyme, or serum proteins such as albumin. An increase in total proteins might be indicative of altered vascular permeability, glandular stimulation, or irritation and injury of the conjunctival lining.

3. Differential cell counts of polymorphonuclear leukocytes (PMNs), macrophages, and epithelial cells. Cell counts signal immune changes in inflammatory responses.

Eight subjects were studied. Two were laboratory personnel; two were volunteers who stated they secreted copious tear fluid upon contact with irritant vapors such as are released by freshly crushed onions. Four were subjects with a diagnosis of multiple chemical sensitivities (MCS). Samples were obtained under three circumstances:
(1) Before and after a 45 min exposure to room air using the goggle system or after a 75-120 min exposure to clean, filtered air in the whole body chamber,

(2) Before and after a 45 min exposure to approximately .15 ppm of acrolein (administered through goggles),

(3) Before and after a 75-120 min exposure in a whole body chamber to a mixture of toluene, m-xylene, and n-hexane, combined in equal concentrations totalling less than 20 mg/m^3 total) and lasting up to 2 hr.

In all of the above circumstances, we collected tear fluid immediately following the exposure and 1 hr later.

Results and commentary: The volume of tear fluid collected from a single eye ranged from .05 to 89.60 mg with a mean of 15.43 mg, based on 75 measurements. The mean for the pooled specimens (both eyes) ranged from 0.80 to 122.8 with a mean of 27.59 mg, based on 30 observations. Figure 2 shows mean changes from baseline in total protein and LDH following exposures.

a. Control values (room air, goggles, filtered air, chamber). Two subjects reported slight increases in eye irritation following exposure to filtered air.

b. Acrolein. Four subjects (assays lost to a laboratory accident in two cases) reported increased eye irritation following exposure to 150 ppb of acrolein. Following acrolein exposure, there was a suggestive increase in total protein in the two subjects for whom measurements were available, but no obvious trend. There was no associated change in LDH after acrolein despite irritation symptoms that were severe.

c. VOC. One out of six subjects reported eye irritation following exposure to mixed VOC (toluene, m-xylene, n-hexane mixed in equal concentrations at less than 20 mg/m^3 total concentration). There was no change in total protein or in LDH following VOC exposure compared with clean, filtered air or room air exposure.

There was no evidence of PMNs or macrophages in tear fluid before or after any exposure. Epithelial cells (singly, not in sheets) were present in most of the control tear fluid samples as well as following exposures to acrolein and VOC. To what extent the epithelial cells resulted from friction associated with the tear fluid collection procedure is uncertain. Because cells were found singly rather than in sheets, it is possible that they may be residents of normal tear fluid.

Although symptoms were not significant predictors of changes in total proteins or LDH, the most intense symptom reports were associated with apparent increases in both total proteins and LDH. Total proteins may be a more sensitive index of eye irritation than LDH based on these findings; moreover, an analysis of specific components might prove to be incisive. We did not determine the specific source of the increases in total protein.

We believe that information on factors contributing to the variability found in these assays of tear fluid, some of which are probably technical rather than biological, is needed before the procedure is applied to studies of eye irritation.

5a. The effects of VOC on neurobehavioral performance and subjective complaints.

Rationale: The chamber analogue was designed to simulate a Sick Building exposure. It emphasized collecting information about subjective complaints that are associated with SBS, especially eye irritation and symptoms related to the nervous system (NS) and upper respiratory tract.

Procedures: Six subjects participated in the whole body chamber studies. Four subjects had a history of illness complaints in office buildings and had been diagnosed with MCS; eye complaints were not prominent among their complaints. Two laboratory personnel served as control subjects; they did not have a history of illness complaints in an office building nor did they perceive themselves to be sensitive to multiple chemicals. Their exposures were shortened inadvertently, in one, after 75 min due to a failure of the VOC generating equipment, in the other, after 90 min due to a prior commitment.
The subjects were exposed in a whole body chamber (9 x 7 x 8 ft), lined with stainless steel and featuring temperature, humidity, and contaminant controls.

A mixture of three VOC commonly found in indoor air in homes and in public buildings (Wallace, 1987) was administered. Toluene, m-xylene, and n-hexane were proportioned so that the total exposure level did not exceed 20 mg/m^3 and each substance was less than 1/50 of its industrial occupational exposure limit. A total VOC concentration of 25 mg/m^3 has been used in studies at the Environmental Protection Agency and in Denmark to study Sick Building Syndrome in normal and sensitive subjects.

The VOC generator was a simplified version of that employed by Hudnell et al., 1990 and is shown schematically in Figure 3. The VOC mixture was delivered by means of a syringe pump to a gas chromatograph (GC, Perkin Elmer Sigma 4), which used nitrogen as a carrier gas. Initially, n-hexane, toluene, and m-xylene were mixed in equal proportions to produce the desired concentration. A constant volume of the solvent mixture was injected by means of a Harvard Syringe Pump into the vaporizer to achieve the target VOC concentration. Nitrogen from a commercial tank was delivered at a flow rate of 1 $L\text{-}min^{-1}$ to the vaporizer. Nitrogen (flow rate 1 $L\text{-}min^{-1}$) was used as a carrier gas to deliver the organic mixture to a vaporizer. Beyond the vaporizer, the mixture was diluted by nitrogen (15 $L\text{-}min^{-1}$), passed through a coil in the GC oven, heated tubing beyond the oven, to a duct where it was mixed with filtered air (flow rate 40000 $L\text{-}min^{-1}$) before entering the exposure chamber. The temperature of the GC oven was constantly monitored with a thermocouple temperature monitor. Air samples were drawn from the chamber for GC analysis every 8 min.

The VOC generating system was tested for reproducibility over time, at both sitting (3'8") and standing heights (5'6"), and at different locations within the chamber. Steady state concentrations were achieved within 30-45 min and thereafter maintained within 2% of the average value for 90 min. There were no systematic differences in concentration tested horizontally or vertically. (The sitting mean/standing mean was 0.996. Over time, the variability for both sitting and standing concentrations was less than 1%).

The experimental procedure was as follows: On day 1, subjects practiced each neurobehavioral task three times, completed a questionnaire, and signed a consent form. On day 2, subjects changed into cotton clothing that had been laundered in baking soda and vinegar, rated symptoms, and furnished tear samples and exhaled breath samples. Then, they entered the chamber for 75 to 120 min and breathed clean filtered air. During this

period, subjects completed symptoms ratings every 20 min. The questionnaire listed 33 symptoms that could be categorized as representing 5 systems: nervous system (NS, n=13), including questions about emotions and cognitive function; upper airways, including a question about eye irritation (UAW, n=5), lower airways (LAW, n=5), skin (n=5), and systemic, including cardiovascular (n=5). Following the exposure to filtered air, subjects provided another expired breath sample and tear sample, performed neurobehavioral tasks, and completed symptom ratings. On day 3, the same procedures were repeated with exposure to VOC.

To insure their participation in a study, which was regarded as threatening, the subjects with MCS were informed of the exposure schedule so that they knew beforehand whether they would be exposed to filtered air or VOC. In all instances, sense of smell immediately confirmed VOC exposure sessions.

We assessed the feasibility of measuring the effects of indoor air pollutants on mental performance, as measured by three neurobehavioral tasks of the Neurobehavioral Evaluation System (NES): symbol digit, switching attention, and grammatical reasoning. Six subjects were recruited for this purpose. Three parameters of intrasubject and group performance over time were of interest: (1) degree of variability, (2) slope, and (3) number of trials required to achieve stability. Five subjects completed 13 trials on alternative versions of the tasks. From inspection of the error curves for two of the three tasks (Figure 4, 5), it was clear that degree of variability and pace of learning varied among the tasks both within subjects and for the group as a whole. Using data on the number of errors per task with repeated performance, we selected two neurobehavioral tasks (switching attention, grammatical reasoning) to use in the chamber studies. Data derived from this component of the feasibility study indicated that, on average, 3 trails should be sufficient to minimize the effects of learning on performance during future exposure studies.

Results and
Commentary: All exposures were to < 20 mg/m^3 of n-hexane, toluene, and m-xylene. The highest concentration was 19.173 mg/m^3 total VOC and the lowest was 12.885 mg/m^3 total VOC. Breath samples obtained before and after exposure to VOC showed an elevation of VOC, but the data were not included in the report.

Symptoms. For all categories except skin, MCS subjects experienced a higher frequency of symptoms than control subjects. The symptoms, normalized to the number of subjects in each group, are plotted in Figure 6. The incidence of CNS symptoms was disproportionately high among MCS subjects: CNS symptoms represented 36% of the items on the questionnaire but constituted 58% of the total number they reported. Average number of symptoms during pre-exposure and exposure periods for both VOC and clean air conditions, normalized for number of subject per group, are shown in Table 1 in both MCS and control subjects. Negative difference scores signified more symptoms pre-exposure compared to during exposure; positive difference scores signified more symptoms during exposure compared to pre-exposure. Symptoms in three categories -- NS, upper airway, and skin

increased from pre-exposure levels during VOC exposure in control subjects. In MCS subjects, symptoms in all five categories (NS, lower airway, upper airway, skin, and systemic) increased from pre-exposure levels during VOC exposure. Similarly, for control subjects, average number of NS and upper airway categories of symptoms elevated during VOC exposure as contrasted to clean air exposure; for control subjects, only symptoms referable to the skin were more in evidence during the filtered air exposure. For the MCS subjects, every category of symptom evaluated during VOC exposure compared to clean air exposure. Figure 7 plots the number of nervous system symptoms during exposure, pre-exposure, and post-exposure periods, normalized for number of subjects per group. The MCS subjects reported more nervous system symptoms per subject at all periods than did the control subjects both during VOC and air exposures. However, for both MCS and control subjects, NS symptoms per subject elevated more during VOC exposure as compared to clean air exposure. Further, for both control and MCS subjects, NS symptom elevations were gradual, becoming more pronounced with time and remaining elevated at 1 hour post-exposure. Figure 8 plots the intensity over time of two common symptoms -- confusion and headache -- for MCS and control subjects, normalized for number of subjects per group, for clean air and VOC conditions. For MCS subjects, the intensity of headaches and confusion elevated over time -- markedly during VOC but not during clean air exposure. For control subjects, the intensity of both symptoms was considerably lower over time; and only headaches elevated during VOC, as compared to air, exposure. Like Figure 7, Figure 8 suggests that reports of symptom intensity elevated gradually for MCS and control subjects, supporting the thesis of Hudnell et al., 1990, i.e., that symptom intensity and number are more than a simple function of odor perception which tended to be more intense immediately after VOC exposure began and to diminish over time, according to anecdotal reports.

Cognitive Testing.

Grammatical Reasoning Task, Response Time: The response time for the grammatical reasoning task tended to decrease slightly with practice trials in both control and sensitive subjects (see Figure 9). The trend was more notable between the first and second trials than between the second and third trials. The response times were considerably slower in the MCS subjects than in the control subjects. Neither group showed an effect of VOC on response time compared to that following filtered air.

Grammatical Reasoning Task, Errors: The MCS subjects showed a learning effect during practice trials that was not evident in the control subjects (Figure 10). The lowest number of errors shown by the MCS subjects was seen following exposure to filtered air (equivalent to a fourth practice trial?). Whether the higher number of errors in this group following exposure to VOC was attributable to the exposure itself or was random was not clear. Replicative studies would be required to make this distinction.

Switching Direction Task, Errors: Neither control nor MCS subjects showed systematic changes with repeated practice (Figure 11). The control subjects showed no difference in error score following exposure to VOC and filtered air. The MCS subjects showed more than twice as many errors following VOC than following filtered air.

Our results with both neurobehavioral tasks (Figures 9, 10, 11) in conjunction with the predominance of symptoms referable to the nervous system (Figure 6) appear to implicate the nervous system, particularly the brain, as a target site for VOC in realistic concentrations. To what extent the findings were influenced by foreknowledge about the nature of the exposure (page 7) or, alternatively, by rapid identification of VOC through odor is problematic. The gradual development of symptoms in number and intensity in the MCS subjects following VOC, peaking at a time when odor intensity was likely to be reduced (Figure 7), is not supportive of the sensory hypothesis.

5b Reproducibilities of a Dual-Detector Probe System for Monitoring Metabolism in the Central Nervous System

Rationale: The objective was to validate and assess the usefulness of a simple non-invasive coincidence detector system (dual probe) for the measurement of glucose metabolism in the brain using ^{18}F-deoxy-glucose (^{18}F-DG). These preliminary studies were undertaken to determine the applicability of the dual-probe system for studying the effects of VOC exposure and other inhaled pollutants on regional glucose metabolism in the human brain.

Compared to conventional PET, a dual detector probe permits positron emission studies of the brain at a much reduced radiation dose and cost. Because individuals with SBS complain of symptoms that may be related to central nervous system dysfunction, it seemed plausible to develop an assay of brain metabolism.

Procedures: A simple dual detector system consisting of two sodium iodide scintillator crystals (7.6 cm x 7.6 cm) in cylindrical lead collimators was placed on opposite sides of the subject's head to measure positron annihilation radiation. The separation between the ends of the two collimators was 19.6 cm, with the scintillation crystals being recessed 2.5 cm from the collimator face. By the coincident detection of annihilation gamma rays, tracer radioactivity was measured within specific regions of the brain viewed by the detectors. The slope of the time-radioactivity curve reflected the kinetics of the positron emitting radiotracer. A thermoplastic mask custom fit to the subject's head was used to identify probe positions and regions of interest in the scans. A focused light alignment system allowed accurate positioning of the probe relative to anatomic markers.

Informed consent was obtained on two subjects who fasted 8 to 10 hr prior to each study. The subject's mask was marked to ensure positioning the probe relative to the caudate nuclei and the anterior frontal cortex during the study. A two-dimensional coordinate system was developed with one axis defined as the line which intersects the canthus and the center of the external auditory meatus. The orthogonal coordinate to this line was defined as a line perpendicular to the first line and passing through the center of the external auditory meatus. All probe positions in this plane were expressed in terms of this coordinates system. A focused-light alignment system was used to facilitate these positioning measures, to determine accurate probe positioning over selected regions, and to mark accurately the subject's mask. Measurements were made over 67 and 100 min periods.

The probe was placed in each position shown in Figure 12. The subject lay on a stretcher with her/his head centered between the detectors. The radiotracer was injected into an arm vein and radiotracer accumulation in each brain region was recorded for approximately 10 min. Blood samples (0.5-1.0 ml) were collected from the contralateral dorsal hand vein for the first 60 min of the study to determine the blood clearance of ^{18}F-DG. Five separate blood samples were obtained throughout the study to determine endogenous levels of glucose and to relate blood levels to the rate of increase in the radiotracer in the specific brain regions.

A repeat study was performed about a week later to assess intrasubject assay variability. It was not possible to perform a second study on study day 1 (as in a before-after exposure sequence) because ^{18}F-DG decays with a 110 min. half life.

Results and Commentary: The radioactivity measurements were corrected for random coincidences, system deadtime, and radioactive decay of ^{18}F. The time-activity curves were normalized to the subject's weight and amount of radiotracer injected. The blood ^{18}F radioactivity curve and endogenous levels of blood glucose were used to normalize values for brain glucose metabolism.

As shown in Figures 13 and 14, Subject 1 showed little variability in results with repeated measurements compared to subject 2. However, the procedure itself was found to be excessively confining and uncomfortable as the subject was immobilized for 100 min.

The study had been done with a single bolus injection at the beginning. In an attempt to reduce the time required for the assay and improve reproducibility, the procedure was modified by following the initial bolus injection with continuous infusion of the radiotracer. The results are shown in Figure 15. The slope of the curve was essentially constant and rectilinear by about 35 min (2100 sec), suggesting that the procedure can be shorted, a practical consideration. Observations on additional subjects are in progress.

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Legends

Figure 1: Undiluted acrolein (1 ppm) was stored in a commercial tank and was mixed with commercially purified air. Pressure regulators controlled flow from each source. Flow rate was monitored with meters A and B. The acrolein sampling ports (not shown) were located proximal to both the by-pass valve and goggles. The by-pass valve directed flow to the vacuum until the desired concentration of acrolein was achieved. Thereafter, flow was directed through the goggles. The schematic shows gas flow directed to the goggles. The goggles were evacuated by vacuum through two ports located on the inferior border; evacuation flow was monitored by meter C and balanced against flow into the goggles by a regulator proximal to meter C.

Figure 2: There was a suggestive increase in total protein following acrolein exposure as compared to baseline and to the filtered air condition without an associated change in LDH. VOC exposure did not elicit an increase in either total proteins or LDH above that seen after filtered air.

Figure 3: The VOC mixture was delivered with a Harvard syringe pump through a vaporizer located within a gas chromatograph oven. Nitrogen, preheated through brass tubing, was measured and regulated by two meters. One meter, set at 1 L-min^{-1}, controlled nitrogen to the vaporizer; the other, set at 15 L-min^{-1}, controlled the nitrogen used to dilute the vaporized VOC. The thermocouple was used to monitor the GC oven temperature. The vaporized, diluted nitrogen VOC mixture left the GC oven through stainless steel tubing which was heated (tapeheater, not shown) and was subdivided into three heated streams in a conduit, to reduce back pressure and promote mixing before entering the whole body chamber. Flow rate through the conduit was about 180 cu ft-min^{-1}. The mixture was delivered to the chamber through a single orifice with at a rate of 4000 L-min^{-1}

Figure 4: Stability in terms of number of errors was achieved in 4 out of 5 subjects by the second trial. Similar results were seen with the Symbol Digit Task (not shown).

Figure 5: Only one subject showed obvious learning. The number of errors per trial was approximately twice as great on this task compared to the other neurobehavioral tasks.

Figure 6: Symptoms were more frequent during exposure to VOC than during filtered air, with the exception of symptoms referable to the skin. Symptoms referable to the NS, including those concerned with emotion, cognition, and attentiveness changes, were more frequent than for all other organ systems combined. Symptoms referable to the NS represented about a third of the number specified in the questionnaire, nonetheless more than half of those identified. The mean number of complaints per MCS subject was more than twice as high as in the control subjects.

Figure 7: Mean number of NS symptoms increased gradually over time, more steeply during VOC exposure than during filtered air exposure. MCS subjects experienced more than twice as many NS symptoms as controls during both VOC and filtered air exposures. Difference between mean number of NS symptoms experienced during VOC relative to filtered air exposure was higher in MCS subjects than in controls.

Figure 8: Intensity of symptom ratings tended to increase gradually over time during VOC, but not during filtered air, exposures. MCS subjects reported increased intensity of both confusion and headache during VOC exposure whereas controls reported increased intensity of headache only.

Figure 9: Curves showed a slight reduction in response time with repeated practice trials on two difficult subtypes of items. False component items were those on which the subject had to judge the accuracy of a false statement (i.e., b follows a: ba); passive component items were those in which the subject had to judge the accuracy of a passive statement (i.e., b is not followed by a: ab). See text for details.

Figure 10: Control subjects showed no clear practice (learning) effects and no errors following VOC exposure. MCS subjects showed a trend toward reduced errors with practice. After VOC, their errors exceeded in number those observed after exposure to filtered air and to practice trials 2 and 3.

Figure 11: Neither control nor MCS subjects showed systematic changes with repeated practice. The control subjects showed no difference in error score following exposure to VOC and filtered air. The MCS subjects showed more than twice as many errors following VOC than following filtered air.

Figure 12: Brain regions assessed with the probe. Acquisition periods lasted 5 min.

Figure 13: The results obtained from two brain areas, the caudate and anterior frontal cortex, are shown. Counts per second (a measure of radioligand decay) per dose of ^{18}F-deoxy-glucose was reproducible in subject 1 after a one week interval. All CPS were corrected at the time of dose injection (injected dose = 121.5 μCi in study # 1, 242.0 μCi in study # 2.

Figure 14: Results for the same two brain areas, the caudate and anterior frontal cortex, expressed as CPS per dose of ^{18}F-deoxy-glucose, were less reproducible in Subject 2 than in Subject 1 after a one week interval. The injected doses were 239.4 μCi in study # 1 and 139.4 μCi in study # 2, respectively.

Figure 15: In subject 3, the initial intravenous bolus of ^{18}F-deoxy-glucose was followed by continuous infusion of the radiotracer. High reproducibility was shown by two studies separated by 1 wk. Black dots were obtained during repositioning of the head.

FIGURE 1: GAS DELIVERY SYSTEM FOR EYE EXPOSURE

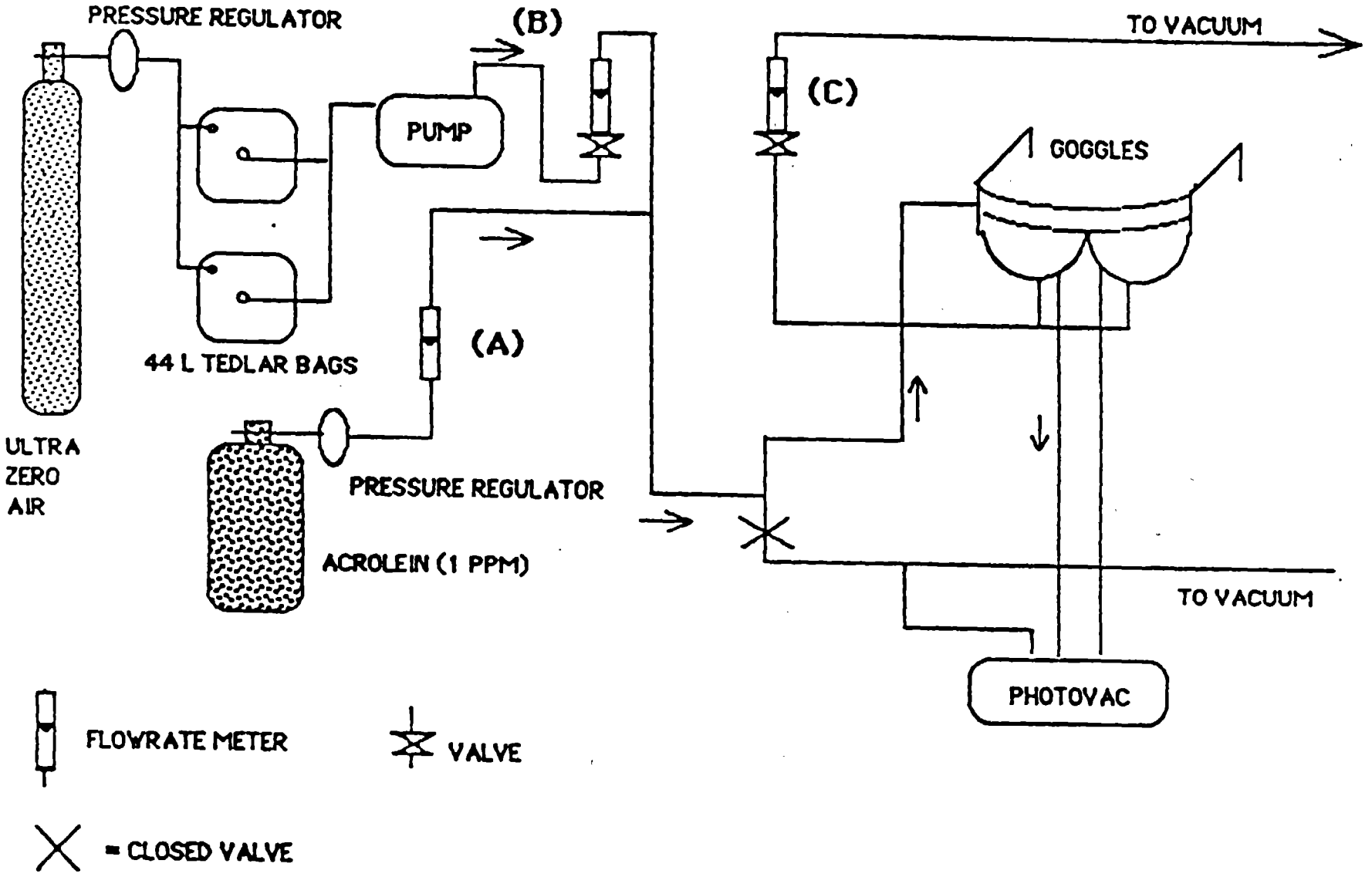


FIGURE 2: TEAR FLUID: TOTAL PROTEIN, LDH

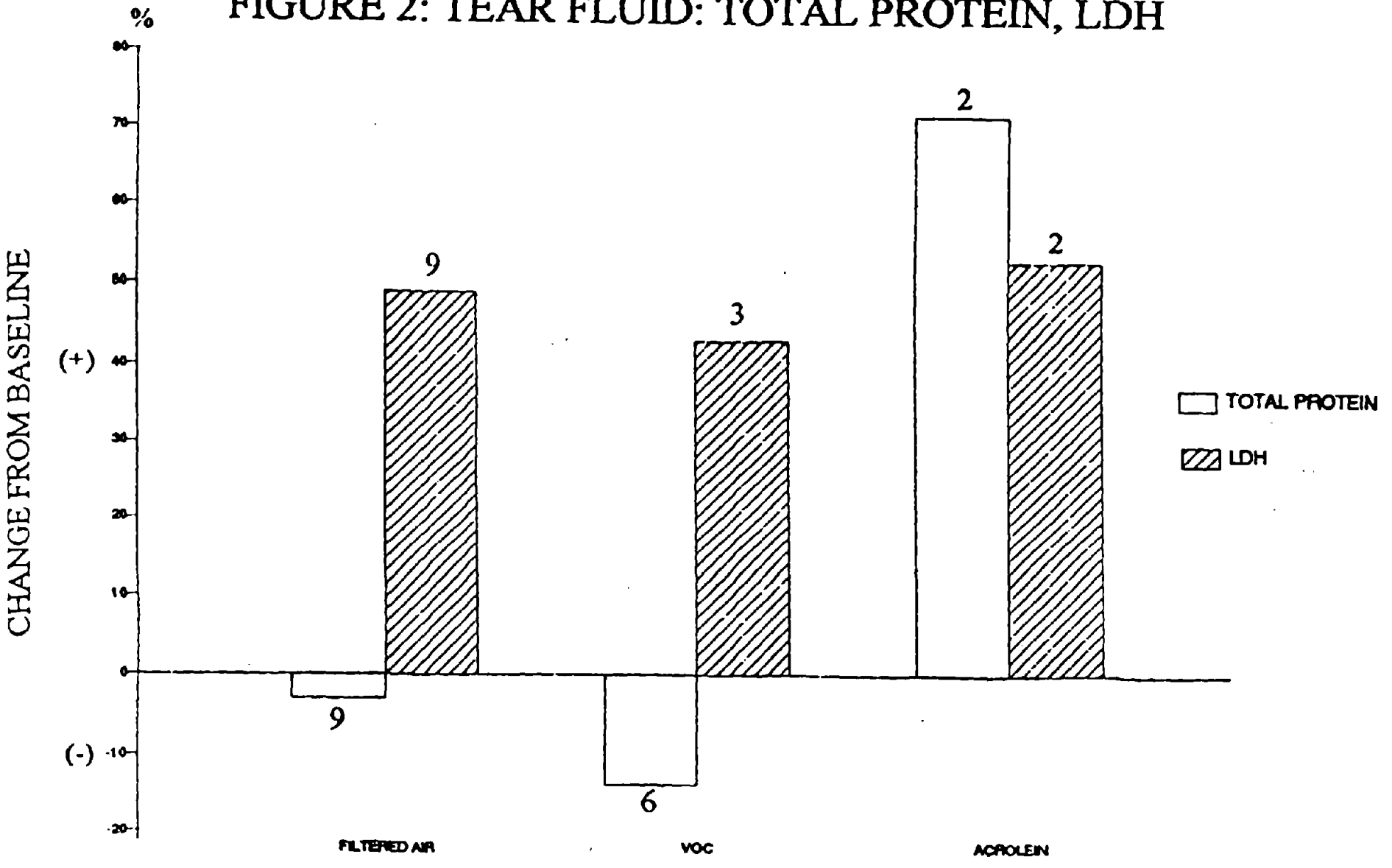


FIGURE 3: CHAMBER VOC GENERATING SYSTEM: WHOLE BODY CHAMBER

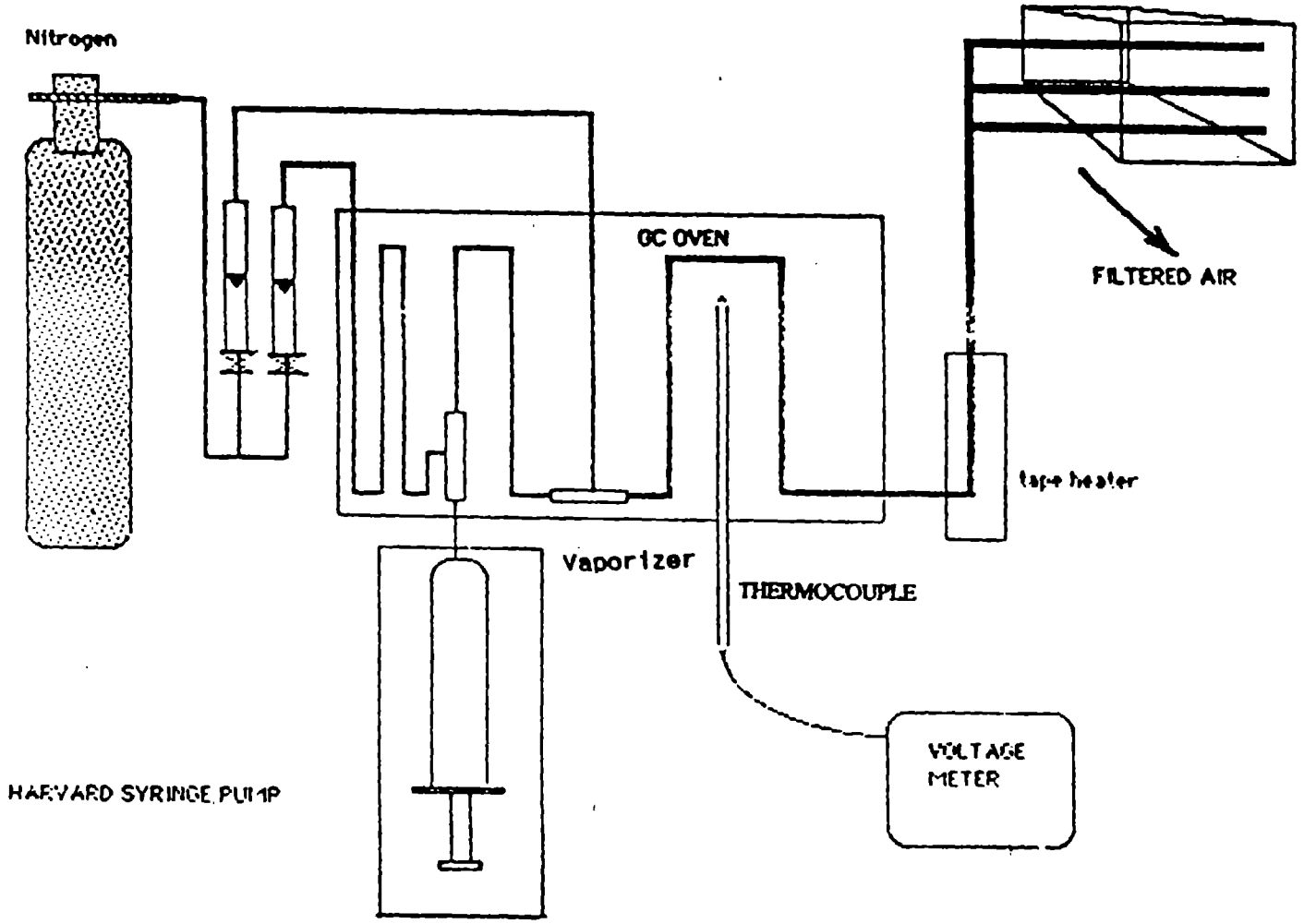


FIGURE 4: LEARNING CURVES, GRAMMATICAL REASONING TASK (5 SUBJECTS)

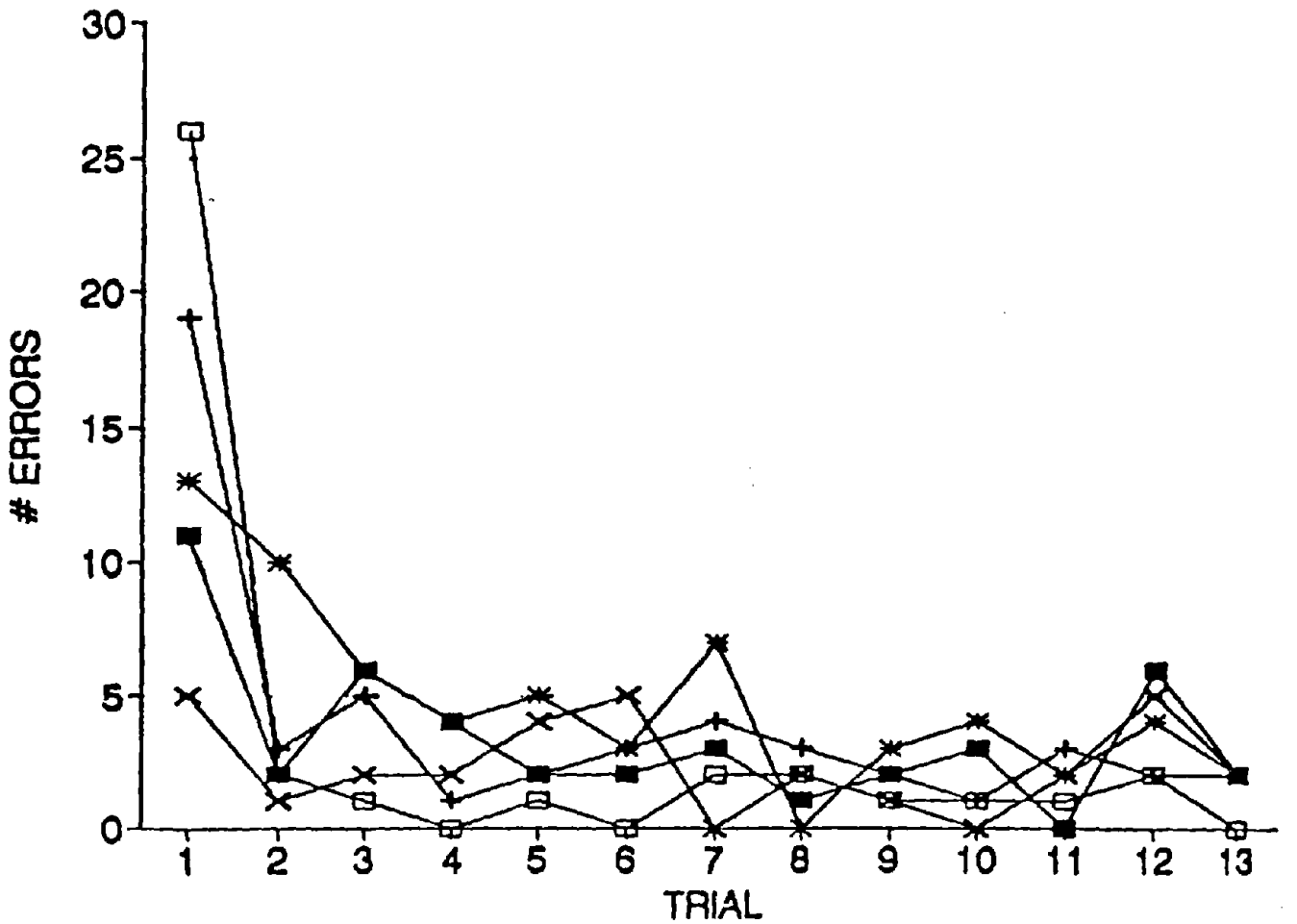


FIGURE 5: LEARNING CURVES, DIRECTIONAL SWITCHING TASK (5 SUBJECTS)

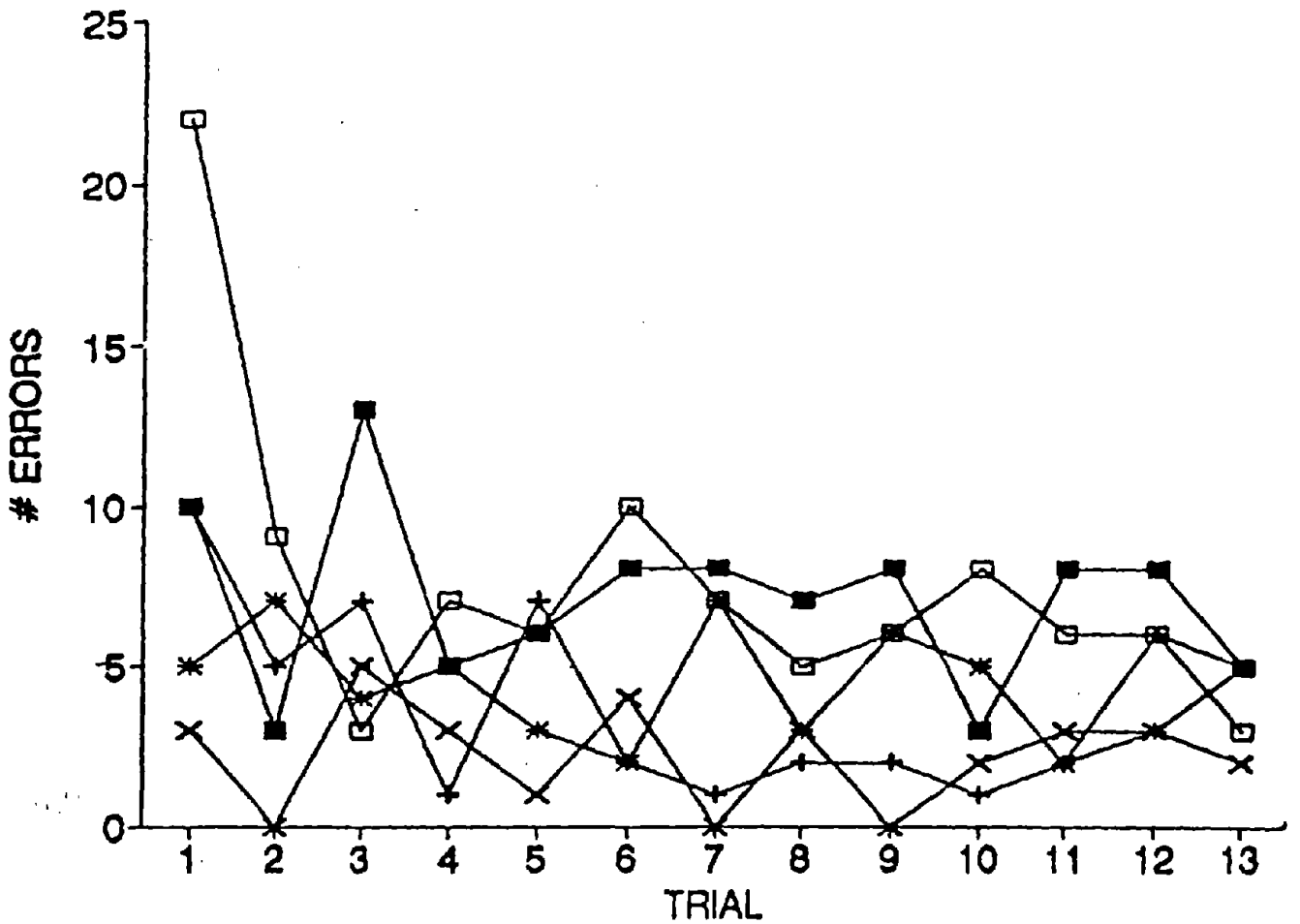
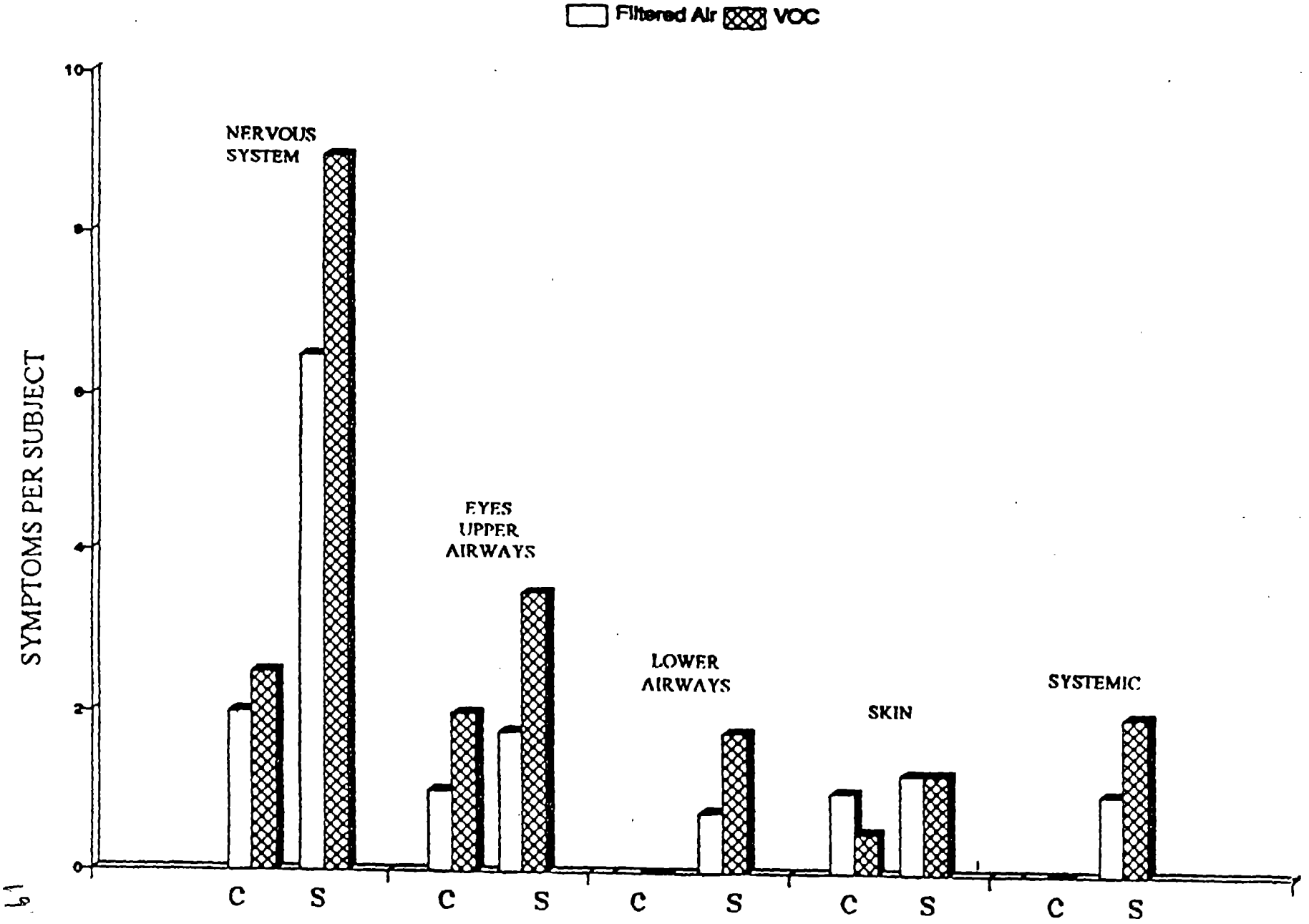


FIGURE 6: SYMPTOM FREQUENCY DURING EXPOSURE



C:: Control Subjects (n=2) S:: Subjects with Multiple Chemical Sensitivities (n=4)

FIGURE 7: TIME COURSE DURING PRE-EXPOSURE, EXPOSURE, POST-EXPOSURE, NERVOUS SYSTEM SYMPTOMS

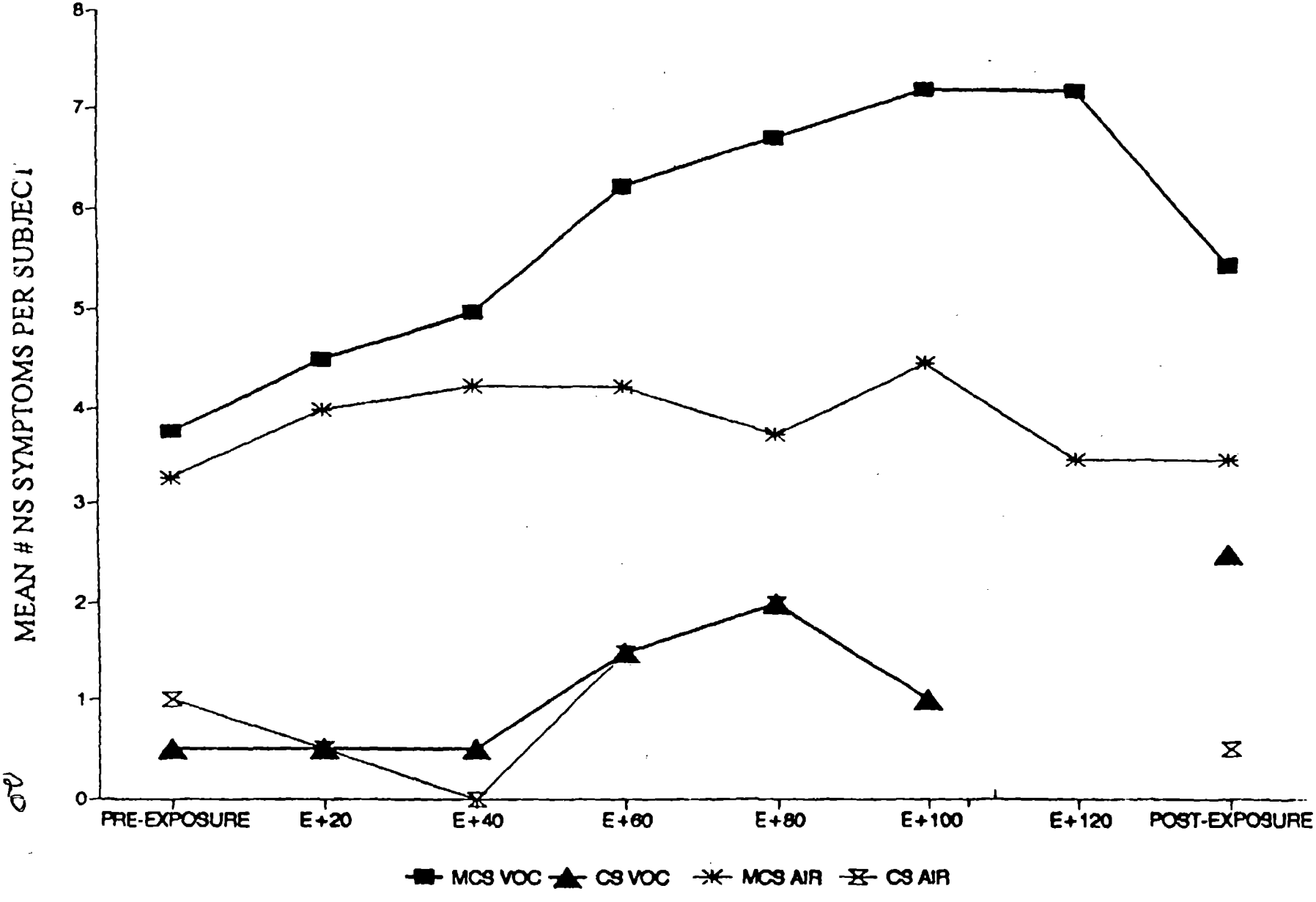
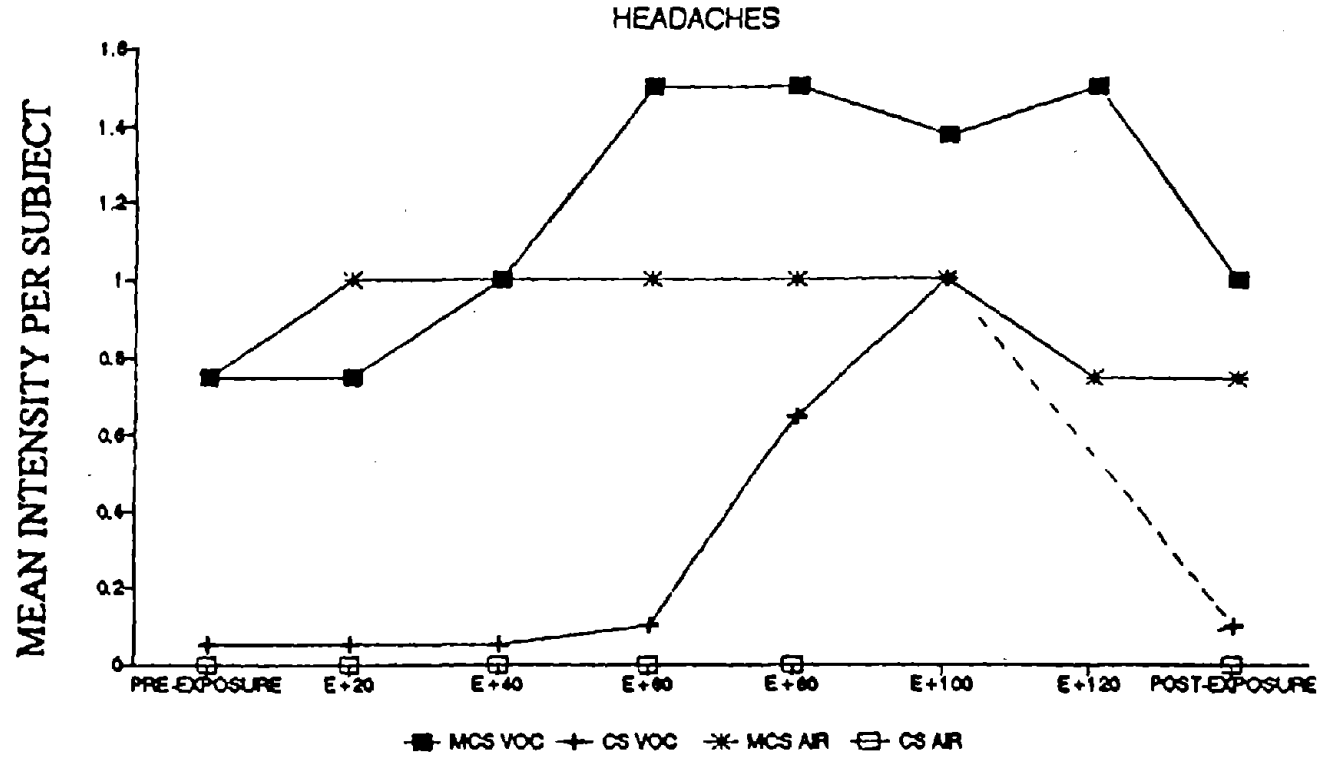


FIGURE 8: INTENSITY DURING EXPOSURE, SELECTED SYMPTOMS



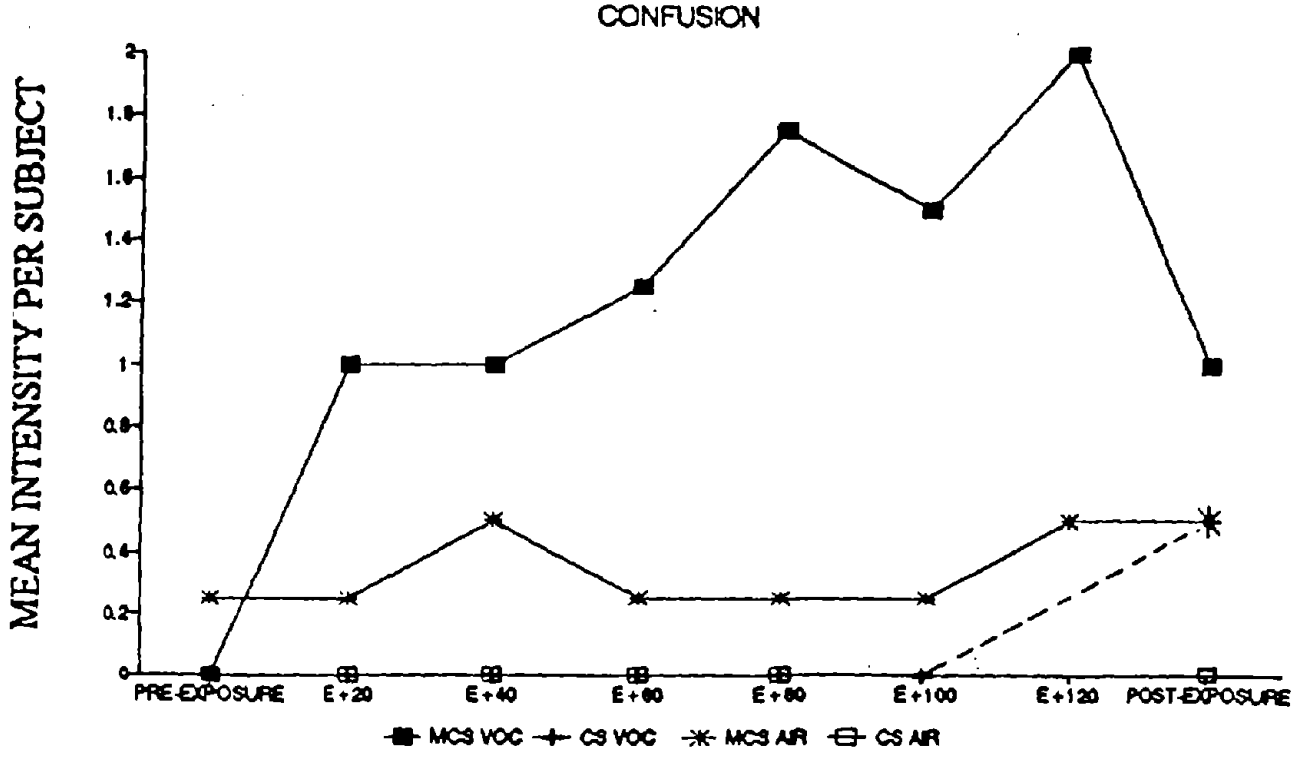


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FIGURE 9: RESPONSE TIME, GRAMMATICAL REASONING TASK FALSE COMPONENT AND PASSIVE COMPONENT

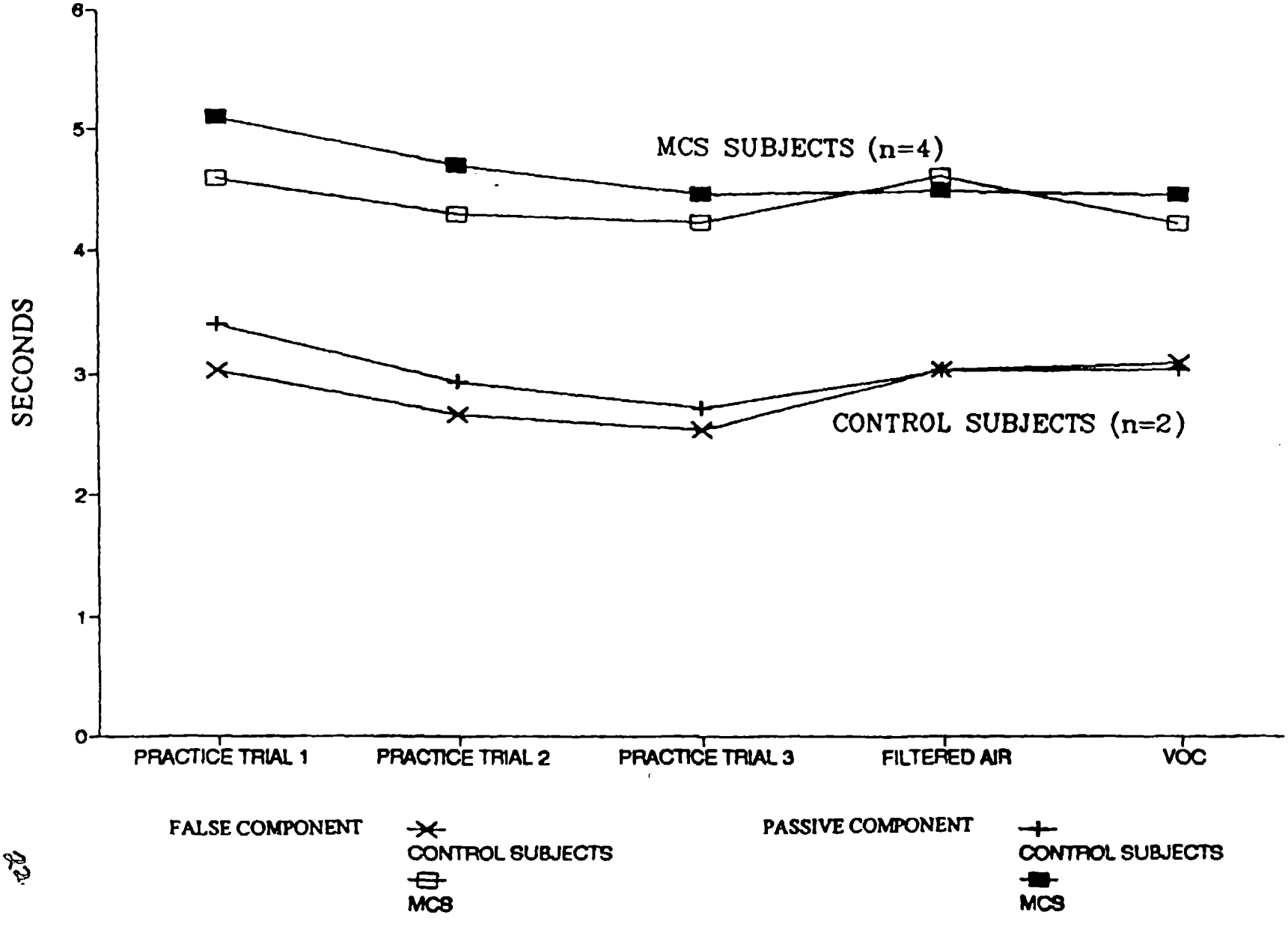


FIGURE 10: ERRORS, GRAMMATICAL REASONING TASK

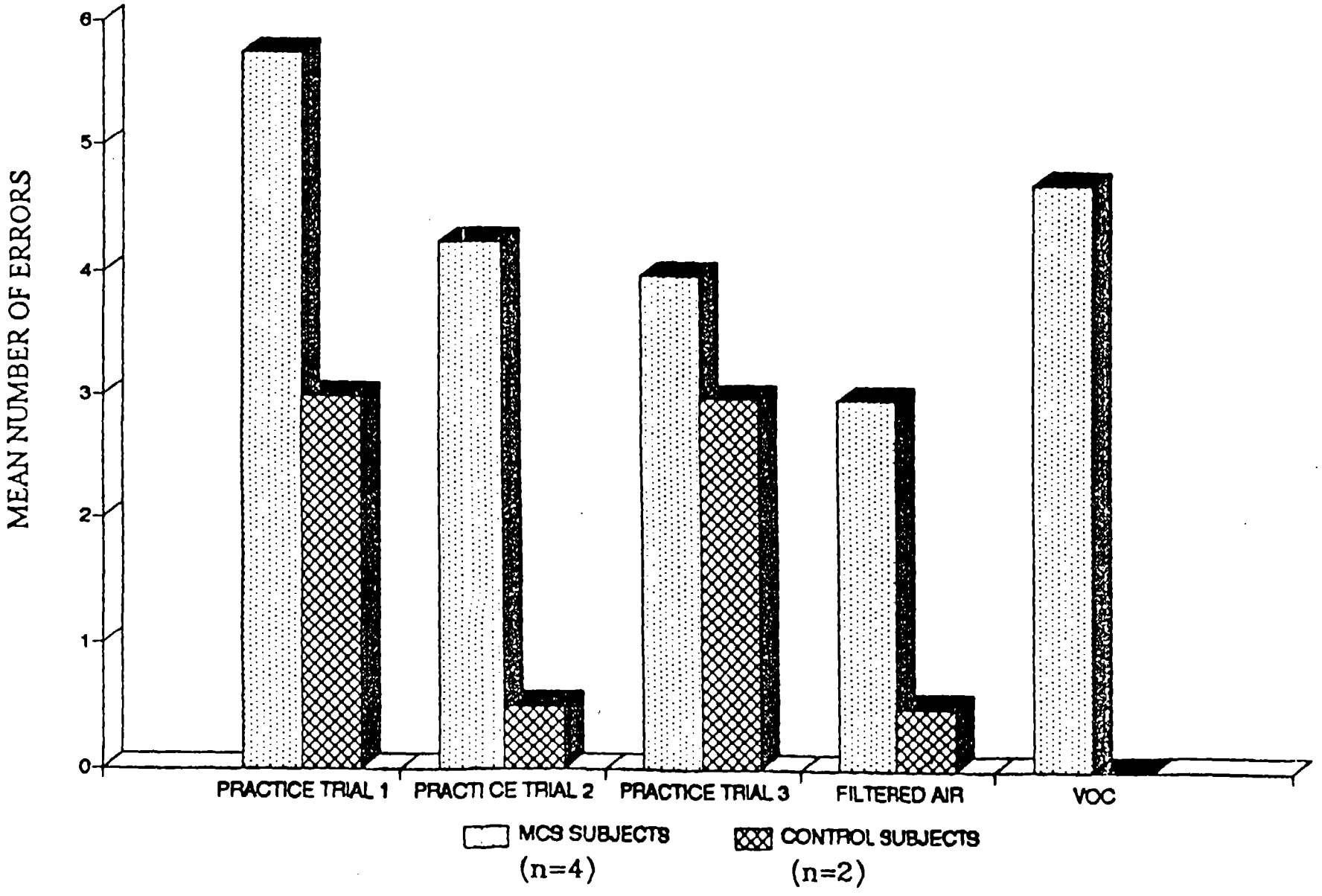


FIGURE 11: ERRORS, SWITCHING DIRECTION TASK

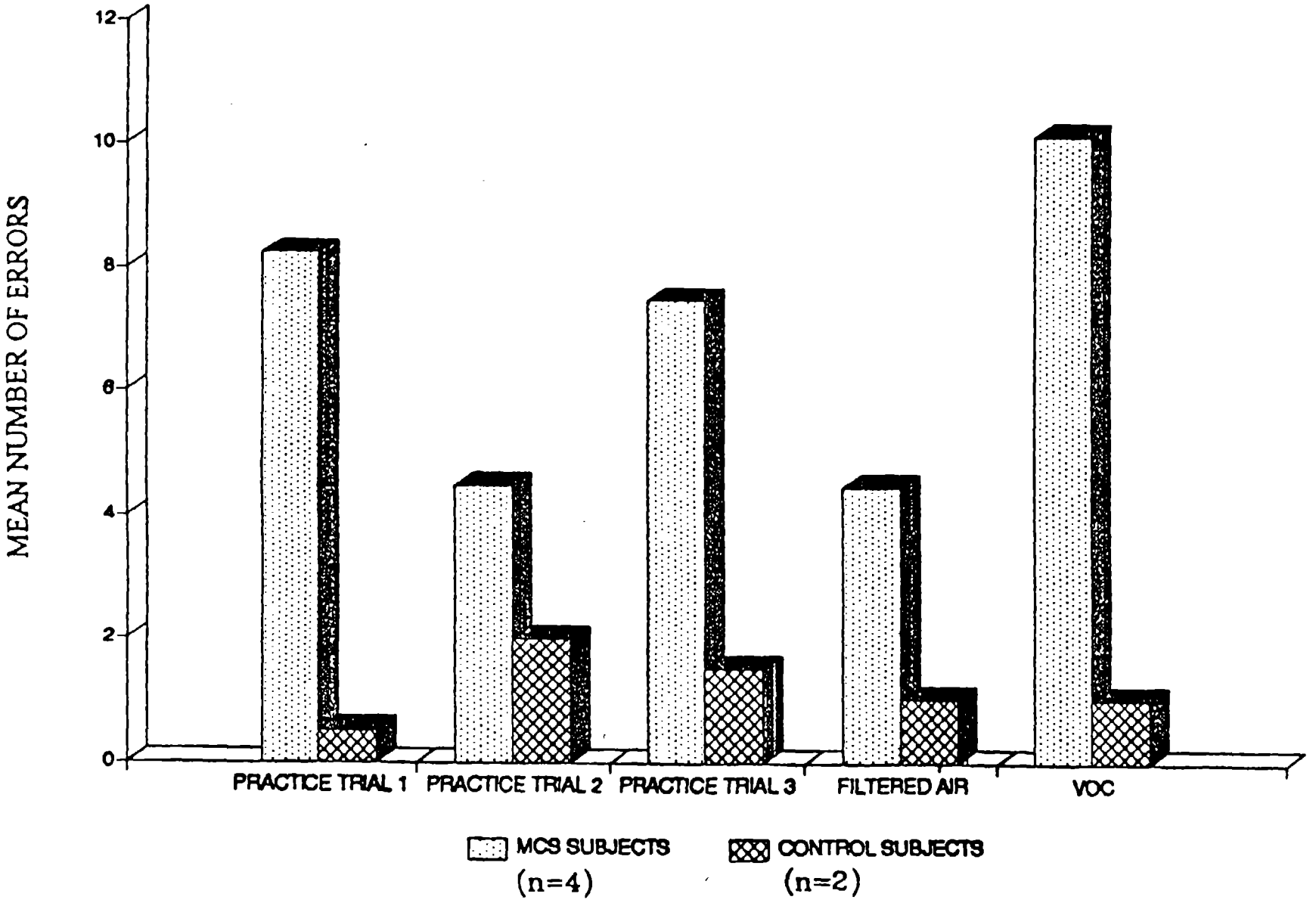


FIGURE 12: BRAIN REGIONS PROBED

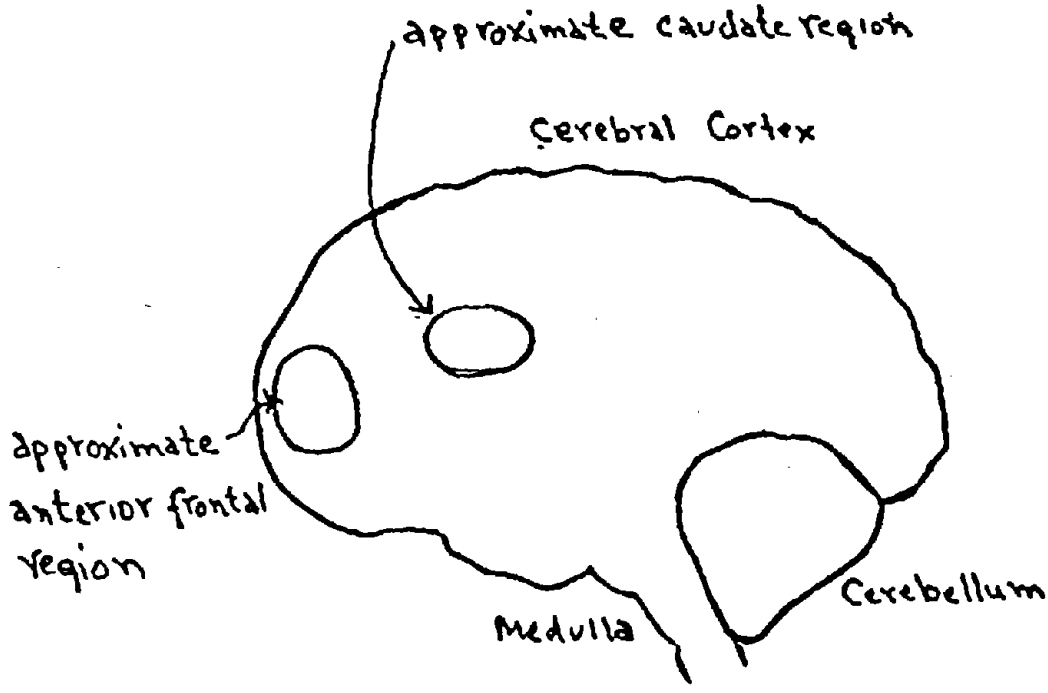
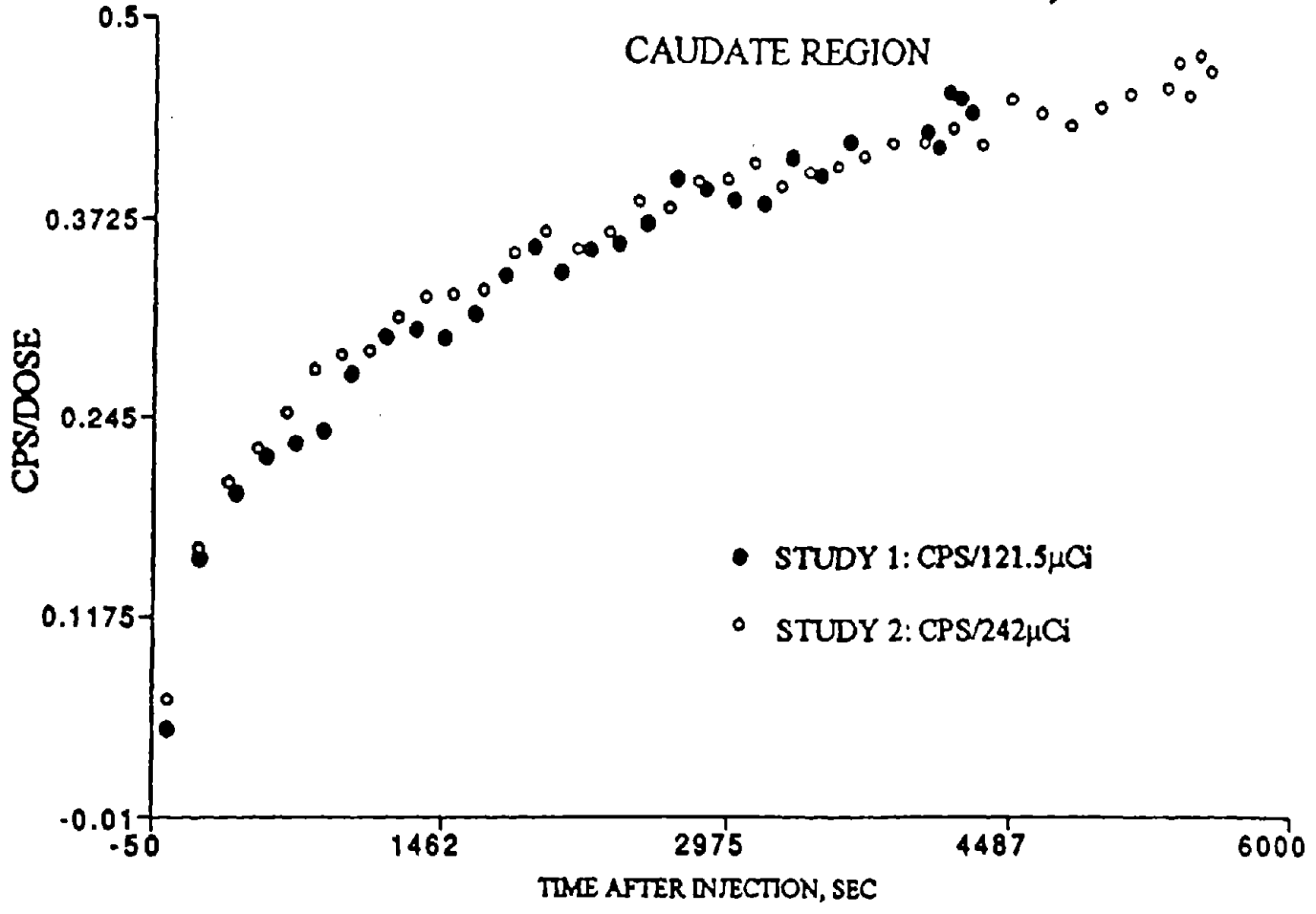


FIGURE 13: 18 FDG RADIO TRACER, SUBJECT I



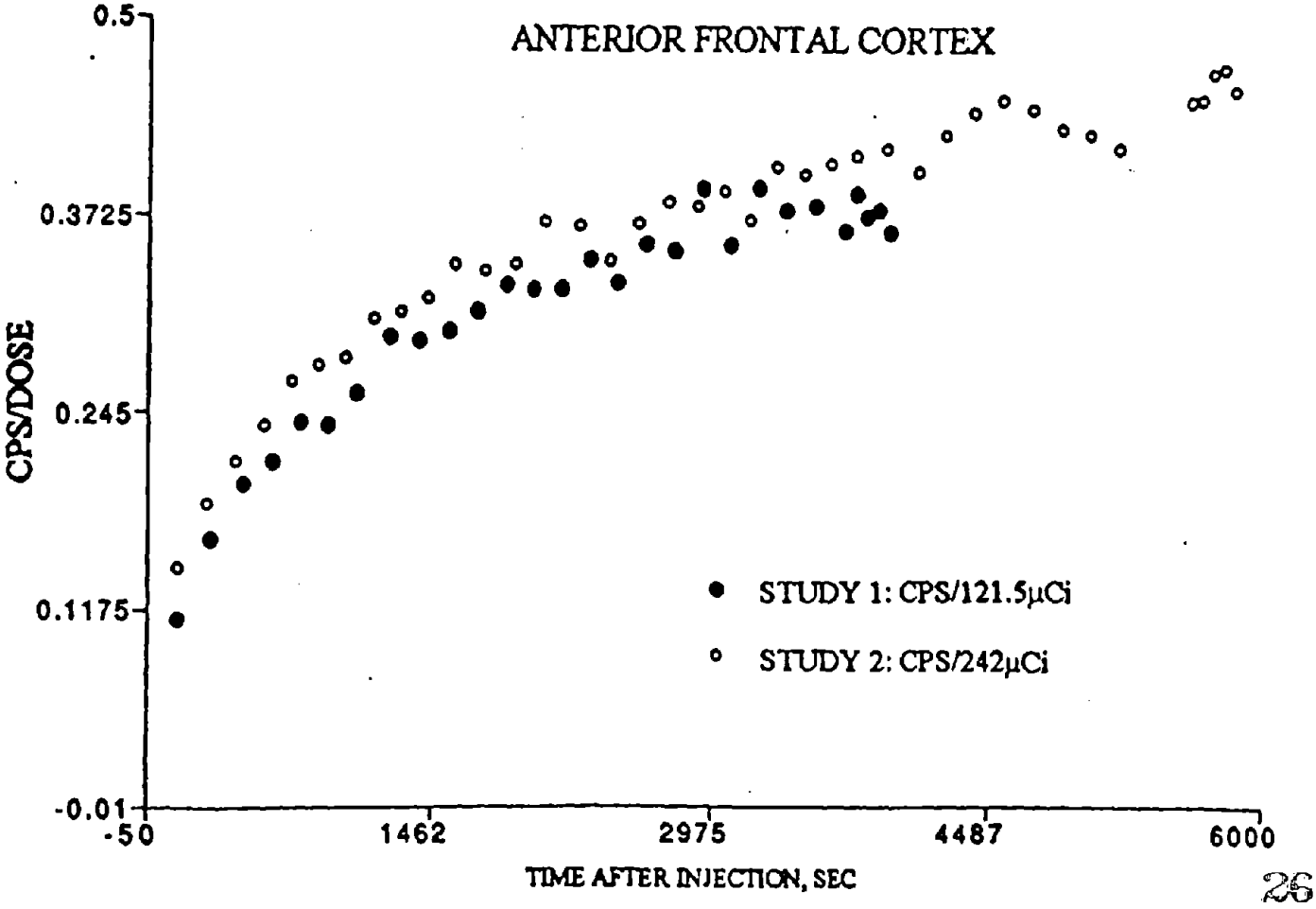
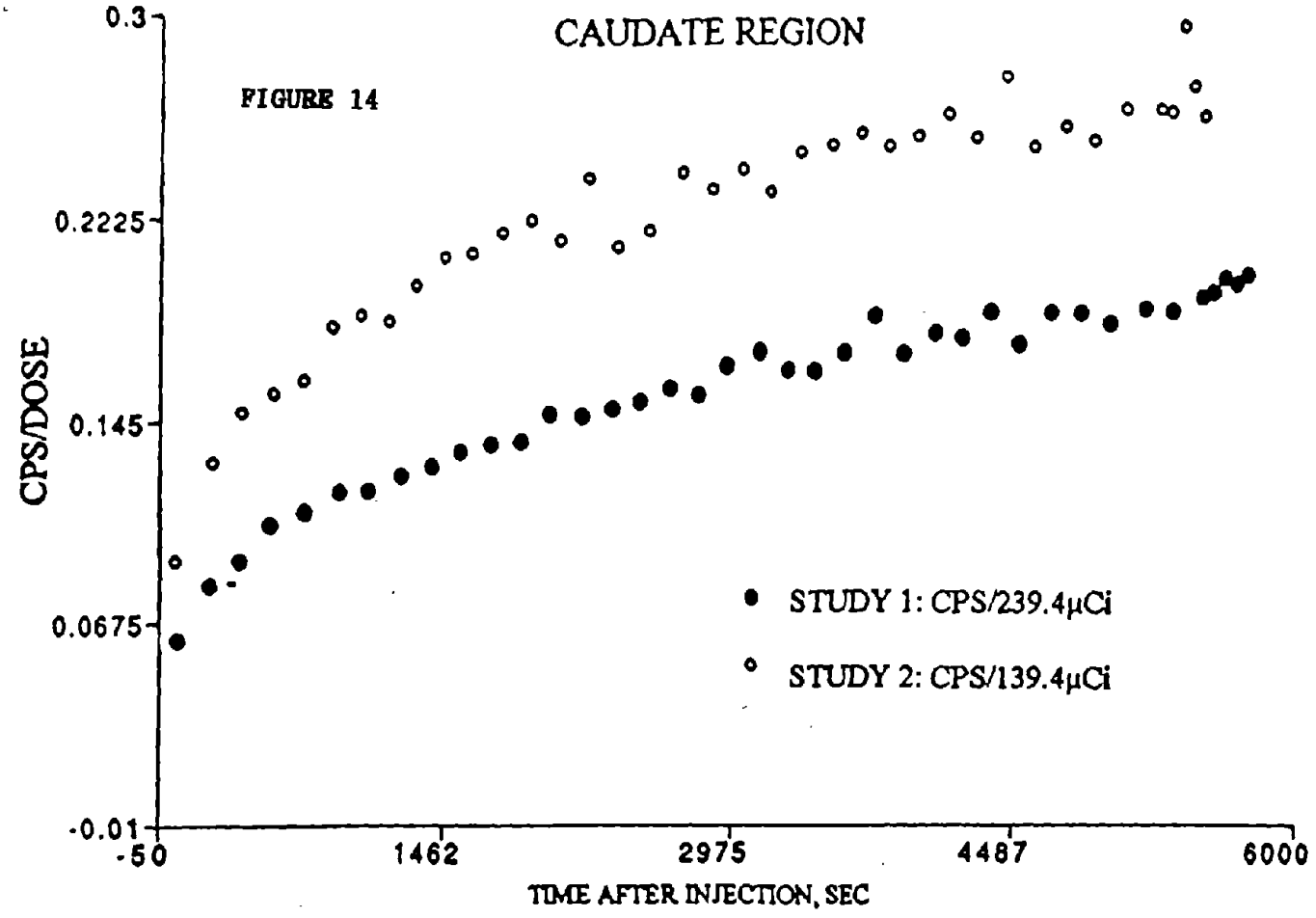


FIGURE 14


CAUDATE REGION
0.3
0.2225
0.145
0.0675
-0.01
CPS/DOSE
-50
1462
2975
4487
6000
TIME AFTER INJECTION, SEC
STUDY 1: CPS/239.4µCi
STUDY 2: CPS/139.4µCi

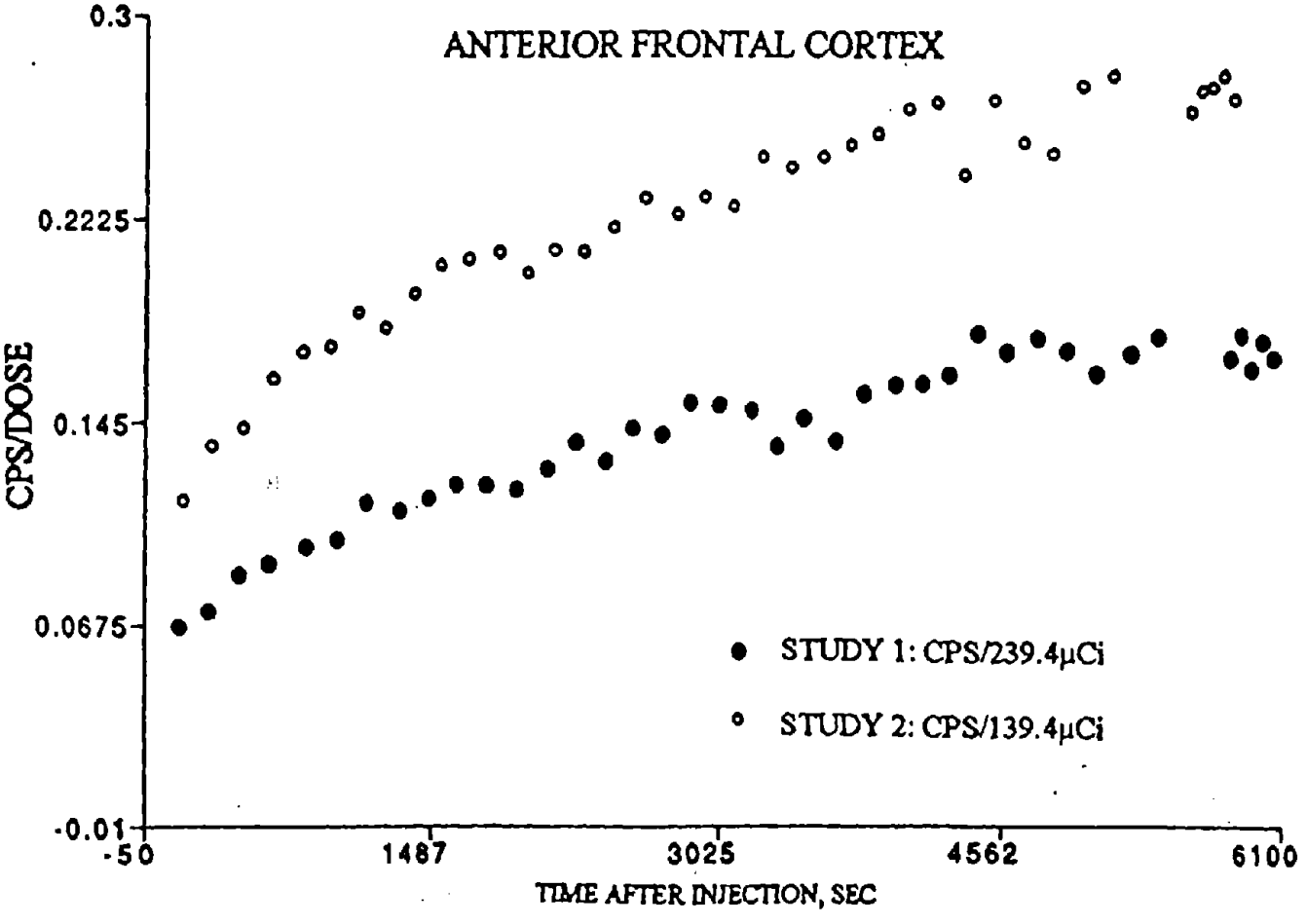

ANTERIOR FRONTAL CORTEX
0.3
0.2225
0.145
0.0675
-0.01
CPS/DOSE
-50
1487
3025
4562
6100
TIME AFTER INJECTION, SEC
STUDY 1: CPS/239.4µCi
STUDY 2: CPS/139.4µCi

FIGURE 15: BOLUS AND CONTINUOUS INFUSION METHOD, SUBJECT 3

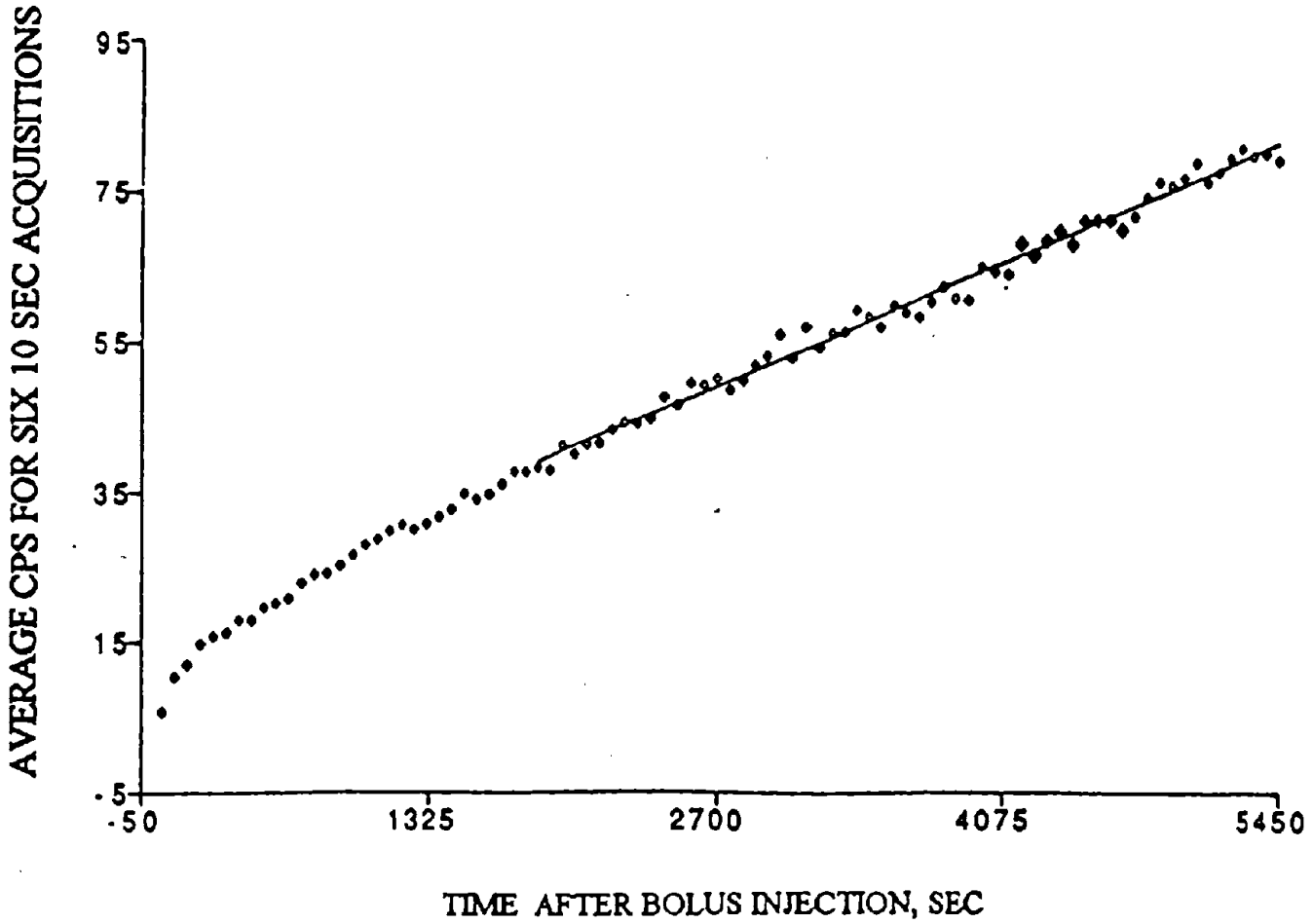


TABLE 1: TOTAL NUMBER OF SYMPTOMS PER SUBJECT BY SYSTEM, ADJUSTED FOR PRE-EXPOSURE VALUES

		NS	LAW	UAW	SKIN	SYSTEMIC
FILTERED AIR	CONTROL SUBJECTS					
	n=2					
	PRE-EXPOSURE	1.00	0.00	0.50	0.00	0.00
	AVERAGE DURING EXPOSURE	0.86	0.00	1.00	0.57	0.00
	DIFFERENCE	-0.14	0.00	0.50	0.57	0.00
	MCS					
	n=4					
	PRE-EXPOSURE	3.25	0.75	2.25	0.75	0.75
	AVERAGE DURING EXPOSURE	4.04	0.33	1.29	1.04	0.83
	DIFFERENCE	0.79	-0.42	-0.96	0.29	0.08
VOC	CONTROL SUBJECTS					
	n=2					
	PRE-EXPOSURE	0.50	0.00	0.00	0.00	0.00
	AVERAGE DURING EXPOSURE	1.11	0.00	1.11	0.11	0.00
	DIFFERENCE	0.61	0.00	1.11	0.11	0.00
	MCS					
	n=4					
	PRE-EXPOSURE	3.75	0.75	1.75	0.25	1.00
	AVERAGE DURING EXPOSURE	6.17	1.33	2.25	0.92	1.13
	DIFFERENCE	2.42	0.58	0.50	0.67	0.13

Appendix

PROGRESS REPORT (INTERIM)

Title:	**Feasibility of a Laboratory Model of Sick Building Syndrome**
PI:	**Robert Frank, MD, Professor, JHU SHPH**
Co-Investigator:	**Linda L. Davidoff, PhD, Associate, JHU SHPH**
Acknowledgement:	**Assistance has been provided by William McArthur, PhD, consultant and Stephan Loh, technician**
Grant Award #:	**90-32**

The past seven months of the contract have been devoted to:

a) Preliminary studies of fractional uptake and excretion of VOC (Specific Aim 1)
b) Developing and testing a technique for correlating the effects of VOC on the composition of tear fluid and symptoms of eye irritation (Specific Aim 3)
c) Testing the effects of learning on the performance of neurobehavioral tasks which will be used in later phases of the research (Specific Aim 5a)

a) <u>Inhalation exposure, fractional uptake and excretion of VOC</u>

Fractional Uptake:
Procedures:

We measured the fractional uptake of VOC in duplicate in 3 subjects following a single breath from a reservoir bag containing 2 VOC. The VOC inhalants were toluene and n-hexane. Relatively high concentrations of the gases were injected into 2 L Tedlar bags, previously filled with 1.5 L of clean air to attain roughly a 10 mg/m^3 combined concentration. The subjects were instructed to inhale directly from the intubated bag at a steady rate equivalent to quiet breathing for 5 s, and immediately thereafter to exhale at approximately the same rate, also for 5 s, into an empty bag. The inhaled and exhaled gas volumes were measured with a large syringe. Before exposure, the subject's expired tidal volume (V_T) and a sample of room air in the subject's breathing zone were collected for analysis of the 2 VOC (Table 1). Each sample was analyzed in triplicate with a Photovac Gas Chromatograph.

Results and Commentary:

N-hexane was not detectable in either room air (background) or baseline expired gas samples. (The limit of detection of the Photovac Gas Chromatograph is reported by the manufacturer to be 50 ppb). Toluene ranged from 79.2 to 129.9 ppb in room air, and from 16.8 to 64.6 ppb in baseline expired gas (Table 2). The limit of detection reported by the manufacturer for toluene with this technique is 5 ppb. The fractional uptakes of the two VOC, calculated as (concentration, expired gas/concentration, inspired gas) x 100, were similar, ranging from 67% to 98% (Table 2). By

comparison, the difference in uptake between the two VOC within each expired gas sample was small, averaging only 3.7%. To gauge the contribution made by alveolar gas to the measurement of uptake, we assumed that the volume of anatomic dead space (V_D) in ml is roughly equal to body weight in pounds and that the percentage of expired gas coming from the alveolar region in each sample equaled $(V_T - V_D)/V_T$ x 100. While the small number of observations argues against drawing any conclusion, it would appear that there was no consistent association between the size of the alveolar component in expired gas and the fractional uptake (or conversely, expired gas VOC concentration). The chief source of VOC in expired gas is reported to come from the alveolar region (Raymer et al., 1990).

Procedure:

We measured total uptake of toluene and n-hexane over a 2 hr exposure period and related these measurements to the amounts excreted by the respiratory system in the post-exposure period. The differences between the two sets of values, assuming respiratory excretion approached zero during the period of measurement, should provide an indication of the capacity of the organism for metabolic transformation of the two pollutants. The exposure system is shown schematically in Figure 1. Toluene and n-hexane were fed into the system in liquid form at a constant rate through a syringe mounted on a Harvard Compact Syringe Pump and were volatilized by heat and mixed with a constant flow ($\sim$250 L/min) of partially filtered, humidified room air. The mixed gases were administered to the subject by means of a weightless head dome, that is supported from above (Bowes et al., 1990). The mixture was vented to the exhaust hood by valve D (shown open to the head dome) until the desired concentrations of the 2 gases were achieved and found to be stable. At that time, the subject donned the head dome. Respiratory frequency (f), V_T and minute ventilation (V_E) were recorded unobtrusively and continuously throughout exposure by means of a heated pneumotachograph (Rudolph) attached to the distal (top) end of a head dome worn by the subject. The calculation of total ventilation was based on ventilation measured at 10 min intervals. Fractional uptake was measured prior to the onset of exposure, following 110 min of continuous exposure and 2 min following the end of exposure by the method described in a). Measurements of the VOC excreted in exhaled air were begun 9 min following the end of exposure and were collected thereafter at approximately 7 min intervals during the first hr, and at approximately 15 min intervals during the second hr. One subject was studied while seated and at rest.

Results and Commentary:

The effects of a 2 hr exposure to n-hexane and toluene on fractional uptake are summarized in Table 3. The background (room) level of n-hexane was not detectable; the background level of toluene was 101.5 ppb. Baseline levels of n-hexane and toluene in expired gas were not detectable and 93.5 ppb, respectively.

Fractional uptake was reduced for each VOC after 110 min of exposure (for n-hexane, the values were 82% pre-exposure and 66% after 110 min of exposure; for toluene, the respective values were 81% and 74%). The depressed fractional uptakes persisted for 2 min into the post exposure period (Table 3). The effect on fractional uptake may have been related in part to an increase in back pressure of the VOC in the liquid lining of the airways and parenchyma.

With regards to toluene, our findings are similar to those other investigators. Fractional uptakes of 75-80% and their depression following a 2-3 hr exposure have been reported by others (Cohr & Stockholm, 1979). In the case of n-hexane, fractional uptake in our study exceeded that reported by other investigators who studied industrial workers exposed to 45-400 ppm (162-1140 mg/m^3) over varying time periods (Brugnone et al., 1980).

Although VOC exposure concentrations in the range that were used for this study (10 mg/m^3 total VOC) are relatively small compared to industrial levels, they are, nonetheless, of interest because exposures of this magnitude are commonly encountered in new and remodeled homes and office buildings (Wallace et al., 1990) and exceed the total VOC concentrations that have been associated with the reporting of symptoms in more than one clinical study ($\leq$ 5 mg/m^3) (Molhave et al., 1986; Molhave et al., 1988) and the hypothesized threshold level for such symptoms ($\sim$ 1 mg/m^3) (Molhave, 1987).

Respiratory excretion of n-hexane was essentially complete at the time the first post-exposure gas sample was collected (9 min); thereafter, there was no evidence of n-hexane in expired gas (Figure 2A). Others studying industrial workers exposed to higher levels of n-hexane have observed biphasic elimination with half times of 14 min and 2.5 hr post-exposure (Nomiyama and Nomiyama, 1974). Toluene concentrations in expired gas above the baseline value were observed for approximately 17 min post-exposure (Figure 2B).

Ninety-eight percent of n-hexane appeared to be transformed into non-volatile metabolites within the 2 hr period of exposure (Table 4). Greater pulmonary elimination of unchanged n-hexane has been observed with industrial workers exposed to high levels over 4 hr (Nomiyama and Nomiyama, 1974). The results obtained with toluene are not readily interpreted. The baseline value of toluene in expired gas, 93.5 ppb, was above the detection limit of the photovac (5 ppb). The value obtained after 110 minutes of continuous exposure was 358.1 ppb (Figure 2B). At the time of the first post-exposure expired gas sample (9 min post exposure), the value had fallen to 110.5 ppb; a level only slightly in excess of the original baseline value; there was a small further reduction in expired gas concentration over the next 100 minutes. This observation is consistent with published reports suggesting biphasic elimination of toluene with half times of .1 hr and 1.82 hr (Raymer et al., 1991). The observation is also consistent with published reports that, following inhalation of toluene, the

major excretory pathways involve rapid oxidation to benzoic acid, which is conjugated with either glycine and excreted as hippuric acid in urine (Doull et al., 1980) or with glucuronic acid and excreted as benzyl glucuronic acid in urine (Haddad and Winchester, 1983). If, for toluene, we assume that the respiratory excretion attributable to the 2 hr exposure was represented by expired gas concentrations in excess of 95 ppb, the original baseline value, then the total mass transformed metabolically, expressed as a percentage of mass retained, was 99%.

In summary, the post exposure excretion of toluene appeared to be biphasic in character; an initial brief phase of rapidly declining excretion lasting minutes, followed by a more prolonged phase of excretion at a low rate (Figures 2B). A rapid short-lived excretory phase from the respiratory system was also observed for n-hexane, but not a slow phase (Figure 2A). The absence of the slow phase may have reflected the limited sensitivity of our analytic technique. These findings suggest that expired gas samples may not provide reliable quantitative information on exposure to these VOC, even in the event of recent intense dosing. Markers of metabolic transformation, such as might be present in urine or blood, might prove more revealing.

b) Eye irritation exposure:

Procedures,
Progress to Dates:

We are attempting to develop a simple, semi-quantitative method of collecting tear fluid adequate in volume for assessing biochemical and cellular components. Ideally, the method should involve little or no excitation of the lacrimal glands and conjunctiva. Once this is established, we can test preliminary the association between symptoms of eye irritation (due to environmental exposure) and changes in the composition of the tear fluid.

The system used to expose the eyes to an irritant is shown schematically in Figure 3. The undiluted test gas (1 ppm, acrolein) is obtained from a commercial tank. Clean air from two 44 L Tedlar bags may be refilled as needed during the study. Flow from the clean air bags is controlled by a pump and mixes with acrolein from the commercial tank to produce the desired concentration at the sampling site proximal to the goggles. During this phase, the excess gas mixture is vented to a vacuum. To begin eye exposure, the gas is directed through the goggles. Flow rate through the goggles is 2 $L\text{-}min^{-1}$; the dead space of the goggles when attached to a subject is approximately 250 ml. The goggles are under slightly positive pressure to reduce the likelihood that the respiratory system will also be exposed; in the event of a leak, a low-speed fan is directed at the breathing zone.

Future plan:

Our plans call for comparing symptoms of eye irritation and possible changes in composition of tear fluid following exposure to 0.1 to 0.2 ppm of acrolein, administered for up to 1 hr. Using the eye exposure system, we have determined that, at a flow of 2 L-min^{-1}, virtually 100% of the desired concentration of acroline can be achieved in the goggles within 1 min. We have been able to maintain a concentration of acrolein, in a trial run of 30 min, at approximately 150 ppb, as measured at 2 sampling sites within the goggles every 3 min (mean: 169.7 $\pm$ SD: 2.4). Negligible leakage into room air was observed.

We have tested and discarded several conventional approaches to the collection of tear fluid. Micropipettes require a trained physician plus equipment that immobilizes the subject's head; moreover, they are effective only if the volume of tear fluid is augmented. (The volume of tear fluid in the normal eye is estimated to be < 1 μl [Farris et al., 1981]). Lacrymators such as freshly-ground onion and horseradish vapors inhaled nasally have proven to be highly variable in their capacity to elicit reflex tearing; in addition,, they have caused eye discomfort and even conjunctival erythema, which might be associated with confounding changes in tear fluid composition. (We may test this possibility, using our present method for comparison). We have not used the Schirmer Tear Test Strips, which can be placed in the conjunctival sac, because of evidence that they may act as a foreign body that induces changes in tear fluid composition (Farris et al., 1981). The method we have evolved is, as follows: a) A thin strip of filter paper, 5-6 layers thick and cut to conform to the curve of the lower eyelid, is held against the previously, cleaned lower lid; b) A known number and weight of droplets of purified water are dropped onto the eye; c) The subject attempts to mix the droplets and tear fluid by closing the eye and rolling the eyeball; d) The excess fluid is collected and weighed. Thus far, we have been able to collect at least 111 mg per eye; the recovery rate of the water droplets has been 59% or better.

We plan to assay for the following markers:

1. Lactic dehydrogenase, an enzyme involved in glycolysis, indicative of aerobic cell metabolism; an increased level in tear fluid would suggest cell damage (cytotoxicity).

2. Lysozyme, an bactericidal enzyme abundant in tear fluid; a change in activity would be indicative of enzymatic degradation or inducibility of enzyme activity. Lower activity would indicate damage to the enzyme; increased activity would indicate increased metabolism. Lysozyme has been linked with irritation due to smog and other irritants (Schuck et al., 1966).

3. Histamine, a neurotransmitter/mediator, released from mast cells in response to damage.

4. PGE_2 and Thromboxane, two prostaglandins involved in the arachidonic acid cascade, having multiple functions. They serve as local chemotactic mediators for vasoconstriction and are involved in many responses to irritating stimulation elsewhere in the body.

5. Differential cell counts of polymorphonuclear leukocytes (PMNs), macrophages, lymphocytes, and epithelial cells. Cells counts signal immune changes. For example, increases in PMNs or neutrophils are indicative of an inflammatory condition.

c) **The effects of learning on neurobehavioral task performance**

The next component of this study will assess the feasibility of measuring the effects of indoor air pollutants on mental performance, assessed by three neurobehavioral tasks. Eight subjects have been recruited for studies of learning on these neurobehavioral tasks. Six subjects have all completed at least 3 out of 13 trials on alternative versions of the tasks. Data derived from this component of the feasibility study will indicate the number of practice trials needed to minimize the effects of learning on performance during future chamber studies.

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15. Supplementary Notes

16. Abstract (Limit: 200 words) The goal of this study was to develop a laboratory model of Sick Building Syndrome (SBS). Preliminary studies were conducted concerning the fractional uptake and excretion of volatile organic compounds (VOCs). Techniques were developed and tested for correlating the effects of VOCs on the composition of tear fluid and symptoms of eye irritation as eye irritation is a frequently reported symptom of SBS. Lactic-dehydrogenase, total proteins, and differential cell counts of polymorphonuclear leukocytes, macrophages, and epithelial cells were studied as markers in tear fluid. The effects of VOCs on the performance of neurobehavioral tasks were also investigated. Subjects were exposed to a total VOC concentration of 20mg/m3 or less in an exposure chamber, and administered cognitive tests, including a grammatical reasoning task and a switching direction task. The test/retest reliability of cerebral glucose metabolism measurements was established for a dual detector probe system for monitoring metabolism in the central nervous system.

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