

Thrombocytopenia and Altered Platelet Kinetics Associated with Prolonged Pulmonary-artery Catheterization in the Dog

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The authors studied platelet kinetics in healthy dogs subjected to pulmonary-artery (Swan-Ganz) catheterization. Ten dogs with a mean weight of 11.0 ± 1.12 kg (SD) were equally divided into experimental and control groups. A fraction of platelets from each dog was harvested, labelled with ^{51}Cr , and reinfused. Daily blood samples for determinations of radioactivity and platelet count were obtained. From the third to fifth day after platelet labelling, experimental dogs underwent catheterization with Swan-Ganz catheters.

During 48 hours following catheterization, platelet counts decreased 64.2 ± 15.2 per cent from baseline. There was no corresponding change in the control dogs. Calculated platelet survival times (based on decay in platelet radioactivity over time) were 102 ± 6.5 hours for the experimental group and 139.8 ± 16.3 hours for controls ($P < 0.01$). Postmortem examination of one sample dog demonstrated thrombus formation along the length of the catheter and in the pulmonary artery.

Significant thrombocytopenia with decreased platelet survival is associated with the use of Swan-Ganz catheters in dogs. The mechanism has not been defined. This observation adds an important consideration to the interpretation of platelet kinetics observed in many pathologic states in animal models. (Key words: Blood; hemostasis; platelets. Complications: thrombocytopenia. Equipment: catheters, Swan-Ganz. Monitoring: pressure, pulmonary arterial.)

THROMBOCYTOPENIA develops frequently in critically ill patients, and often contributes to morbidity and mortality.¹ Flow-directed balloon-tipped (Swan-Ganz) catheters, commonly used in critically ill patients, are known to be thrombogenic in man.^{2,3} To evaluate the kinetics of the platelet consumption associated with these catheters and the extent to which this contributes to thrombocytopenia, we studied healthy dogs subjected to 48 hours of pulmonary-artery catheterization.

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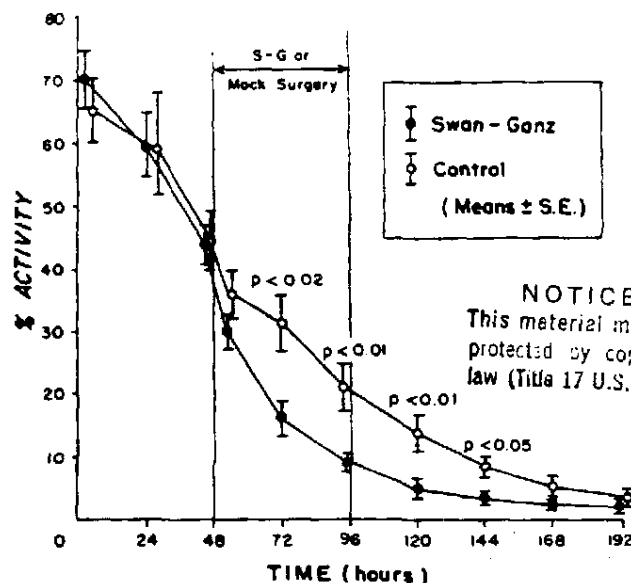


FIG. 1. Composite platelet survival curve based on data from all dogs in the experimental (solid circles) and control (open circles) groups. The steeper slope of the line for the experimental group beginning at the time of catheterization indicates more rapid platelet consumption.

Materials and Methods

Ten healthy male mongrel dogs weighing 9.3–14.1 kg (mean $\pm$ SD: 11.0 ± 1.12) were divided into experimental and control groups; each dog was studied over a period of nine days. On day 1, each dog was anesthetized with thiopental (20 mg/kg), the trachea was intubated, and anesthesia was maintained with halothane (1 per cent) in oxygen. The animals breathed spontaneously at an end-tidal CO_2 of 5 per cent. A total of 90 ml of whole blood was drawn from an external jugular vein into three plastic syringes each containing 5 ml of anticoagulant citrate dextrose solution, USP, formula A.[§] Platelets were labelled with radioactive chromium in the following standard fashion.[¶] The platelet-rich fraction was obtained by differential centrifugation and incubated for one hour with 1.0 mCi ^{51}Cr . The platelets were washed once with platelet-poor plasma, resuspended, and injected. A sample of the injectate was saved for radioactivity

[§] Fenwal Laboratories, Deerfield, Illinois.

[¶] New England Nuclear, Boston, Massachusetts: 1 mCi/ml.

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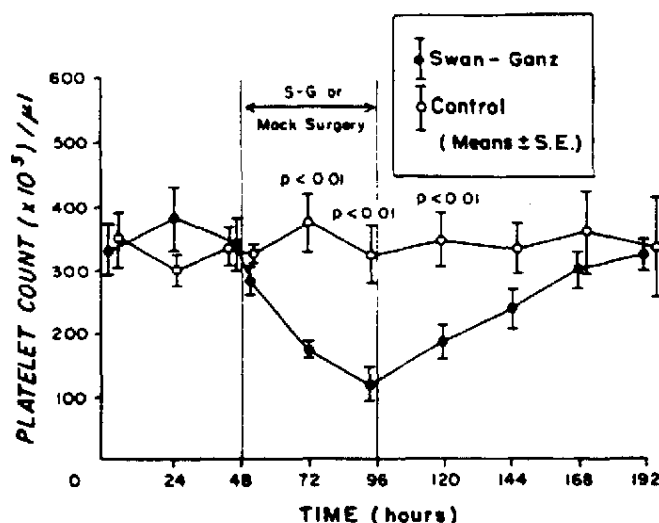


FIG. 2. Mean peripheral blood platelet counts ($\pm$ SE) for experimental (solid circles) and control (open circles) animals. The progressive decline occurring in experimental animals represents a 64.2 ± 15.2 per cent (SD) reduction from baseline, with return toward baseline resulting from removal of the catheter.

counting, and an accurate weight and specific gravity of the injectate were obtained prior to injection. Half an hour after injection, a 3-ml sample of heparinized whole blood was obtained via a peripheral vein for radioactivity counting. Anesthesia was then discontinued, approximately four hours after induction. Every 24 hours thereafter, 3-ml blood samples were taken from a peripheral vein of the awake animal for radioactivity counting. All radioactivity counting was done in triplicate in a gamma well counter.** In addition, we performed daily platelet counts in triplicate by phase-contrast microscopy (method of Brecher *et al.*⁵). On day 3 (48 hours after platelet

labelling), the dogs were again anesthetized with thiopental and halothane; dogs in the experimental group underwent cutdown of the right groin and insertion of a 7-Fr. double-lumen Swan-Ganz catheter†† via the femoral vein, with ligation of the distal femoral vein. The catheter was advanced into the pulmonary artery with confirmation of the final position by recording the pressure tracing on a Grass polygraph (average depth of insertion: 48.8 ± 2.7 cm (SD)). The catheter was flushed with 5 ml of saline solution, 0.9 per cent. The end of the catheter at the groin was occluded with a heavy silk ligature tied tightly at the point of entry of the catheter into the vein, with the remaining catheter external to the vein cut off. The wound was closed and anesthesia discontinued. Dogs in the control group underwent the same anesthetic and surgical procedures for the same length of time (approximately an hour), with ligation of the femoral vein, but without insertion of the catheter. Blood samples for platelet count and radioactivity determinations were obtained six hours after the surgical procedure in addition to the other daily samples. On day 5 (96 hours after platelet labelling), the catheters were removed from the experimental animals, using thiopental-halothane anesthesia; control animals underwent anesthesia without opening and closure of the groin wound, the procedure requiring 45 min for animals in either group. On day 9 (192 hours after platelet labelling), blood volumes of both control and experimental animals were determined by injecting radioactive iodinated (¹³¹I) serum albumin, 0.1 mCi.‡‡ The RISA was diluted, weighed, and counted in the gamma well counter prior to injection; three 1-ml samples of whole blood were obtained for counting half an hour after injection.

** Intertechnique Model 4000.

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TABLE 1. Calculation of Platelet Consumption

	Blood Volume (ml)	Pre-S-G Platelet Count (μ l $\times 10^6$)	Pre-S-G Total Platelets ($\times 10^{11}$)	Post-S-G Platelet Count (μ l $\times 10^6$)	Post-S-G Total Platelets ($\times 10^{11}$)
Experimental Mean $\pm$ SD	970 248.2	3.41 .760	3.35 1.230	1.20 .581	1.28 1.029
Control Mean $\pm$ SD	981 155.8	3.35 .604	3.30 .872	3.25 1.006	3.20 1.230
Significance of difference (experimental vs. control) by Student <i>t</i> test	N.S.	N.S.	N.S.	$P < 0.01$	$P < 0.05$

Rectal temperature was recorded daily. Hematocrit, body weight, and leukocyte count were obtained just prior to and 48 hours after catheterization. Blood cultures were obtained after 48 hours of catheterization.

One additional animal was sacrificed 48 hours after catheterization for observation of clot formation around the catheter and pulmonary artery. This dog received 3000 U heparin, iv, 15 min prior to sacrifice by exsanguination.

The catheter tip from this dog was fixed in 1 per cent glutaraldehyde, dehydrated in graded alcohols and amyl acetate, and finally subjected to critical-point drying. A molecular layer of gold was deposited on the surface and the specimen was examined in a JEOL JSM-50-A scanning electron microscope. Photomicrographs at 1000 $\times$ magnification were obtained.

A segment of left main pulmonary artery from the same dog was fixed in Bouin's solution, sectioned, stained with hematoxylin and eosin, and examined under light microscopy for evidence of thrombus formation.

Decay of platelet radioactivity is expressed by the following equation: Percentage activity at time

$$t = \frac{\text{counts } ^{51}\text{Cr/ml blood at time } t \times \text{blood volume}}{\text{total counts injected initially}} \times 100$$

Plotting percentage activity as a function of time (t) gives a platelet survival (decay) curve; typically, activity immediately after injection of the labelled platelets is about 60 per cent due to initial splenic uptake of injected platelets ("per cent recovery").⁸ Calculation of platelet survival time was done in a standard fashion⁴ using the four points on the platelet survival curve (per cent activity vs. time) obtained just prior to Swan-Ganz catheter insertion and during the two days when the catheter was in place (per cent activity 48, 54, 72, and

96 hours after labelling) in the experimental group, and at the same time intervals in the control group.

Changes in total numbers of platelets for all dogs during the interval of catheterization (experimental) or sham operation (control) were calculated. Total circulating platelets immediately prior to insertion and just before removal of the catheters (or sham operation) was derived by multiplying blood volume times peripheral blood platelet count. The net change in platelets is the difference in total platelets during the interval of catheterization (or between the two sham surgical procedures). The percentage change in radioactive labelled platelet activity during the time of catheterization (or sham operation):

$$\left(\frac{\text{Activity pre-catheterization} - \text{activity post-catheterization}}{\text{Activity pre-catheterization}} \times 100 \right)$$

multiplied by the number of total platelets before catheterization estimates the number of total platelets consumed during that time interval. Effective production is the sum of the total platelets consumed plus the overall net change in total platelets.

Unpaired Student t tests were used to compare means of the two populations, with $P < .05$ considered statistically significant.

Results

The platelet survival curves for the control and experimental animals, with each curve derived from the average percentage radioactivity ($\pm$ SE) for the five dogs in the group, are almost identical in appearance until 48 hours, the time of catheterization in the experimental group (Fig. 1). Thereafter, the survival curve for the experimental group shows a more rapid decay (steeper slope). Mean platelet survival times

Production (S-G = Swan-Ganz Catheterization)

S-G cat lets (n)	Net Total Platelets (Post-S-G - Pre-S-G) ($\times 10^9$)	Per Cent Activity Pre-S-G	Per Cent Activity Post-S-G	(Per Cent Pre-S-G - Per Cent Post-S-G) Per Cent Pre-S-G $\times 100 = \text{Per Cent } \Delta$	Consumption = Per Cent Δ $\times \text{Total Pre-S-G}$ ($\times 10^9$)	Effective Production = Net + Consumption ($\times 10^9$)
18 29	-2.07 .678	43.2 5.81	8.5 3.80	80.3 8.54	2.66 .910	0.59 .671
20 30	-.094 .443	44.6 10.96	21.8 7.60	51.7 8.206	1.70 .456	1.61 1.641
0.05	$P < 0.0005$	N.S.	$P < 0.01$	$P < 0.0005$	$P < 0.05$	$P < 0.05$

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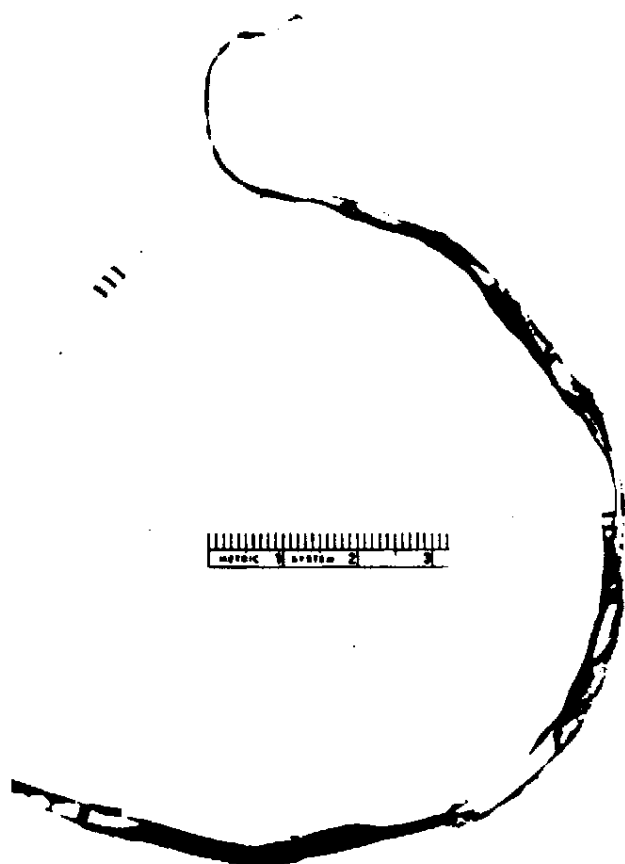


FIG. 3. Organized thrombus surrounding the portion of the catheter that lies in the inferior vena cava, taken after 48 hours of catheterization from a dog systemically heparinized just prior to sacrifice.

calculated from the four points on the curve obtained just prior to and during catheterization were 102 ± 6.5 hours (SD) for the experimental group and 139.8 ± 16.3 hours for the control group, significantly different at $P < 0.01$.

Average peripheral blood platelet counts in the groups were the same at the beginning, but a decrease in the experimental group began to become apparent as early as six hours after catheterization, was significant after 24 hours, and continued until removal of the catheter (fig. 2). The nadir of the curve after 96 hours of catheterization (96 hours after platelet labelling) represents a 64.2 ± 15.2 per cent ($\text{mean} \pm \text{SD}$) decline from the 48-hour baseline value. Within the next four days, platelet counts returned to baseline.

The decrease of total platelets (table 1, column 6) in the experimental group ($2.07 \pm .678 \times 10^{11}$) was significantly larger than that in the control group ($.094 \pm .443 \times 10^{11}$) ($P < 0.0005$). The total number of platelets destroyed (column 10) in the experimental group ($2.66 \pm .910 \times 10^{11}$) was significantly greater than that in the control group ($1.70 \pm .456 \times 10^{11}$). Effective production (column 11) in the experimental group ($.59 \pm .67 \times 10^{11}$) was significantly less than that in the control group ($1.61 \pm 1.641 \times 10^{11}$).

Table 2 shows average body weights, hematocrit, and leukocyte counts for the dogs in both groups just prior to and after 48 hours of catheterization or sham operation. There was no significant difference between the groups. Cultures of blood from all dogs were sterile.

Figure 3 shows organized thrombus surrounding the portion of the catheter that lay in the inferior vena cava of the dog that was systemically heparinized just before sacrifice after 48 hours of catheterization. Thrombus could be seen surrounding the entire length of the catheter, extending into the pulmonary artery, where it was firmly fixed to the arterial wall. The presence of polymorphonuclear leukocytes within the thrombus indicated its age to be at least 12–24 hours. A scanning electron photomicrograph of this catheter surfaced showed deposition of fibrin, erythrocytes, and platelets.

TABLE 2. Changes in Body Weight, Hematocrit, and Leukocyte Count after Catheterization or Sham Operation

	Body Weight (kg)		Hematocrit (Per Cent)		Leukocyte Count ($\times 10^9/\text{min}^3$)	
	Pre-catheterization	Post-catheterization	Pre-catheterization	Post-catheterization	Pre-catheterization	Post-catheterization
Experimental Mean $\pm$ SD	11.0 1.9	11.0 1.9	41.2 2.4	38.8 3.6	9.8 3.9	14.5 3.4
Control Mean $\pm$ SD	10.6 1.0	10.7 1.1	39.4 1.8	37.8 3.1	9.9 3.9	14.2 3.4
Significance of difference (experimental vs. control) by Student <i>t</i> test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

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Discussion

The peripheral blood platelet count represents the balance between platelet production in the marrow and consumption and dilution in the peripheral blood, with production, consumption, and blood volume being constant at steady state.⁶ Thrombocytopenia results from increased platelet consumption (or sequestration), reduced production, or hemodilution. Hemodilution does not explain the large decrease (60 per cent) of platelet counts in our experimental dogs, since other indices of increased plasma or total water volume (i.e., hematocrit and body weight) did not change during catheterization.

Increased platelet consumption is evident from the deeper slope of the ⁵¹Cr platelet survival curve of the catheterized dogs. The absolute magnitude of total platelets consumed is dependent upon the size of the existing pool of circulating platelets, as well as the number of newly formed platelets (ongoing marrow production during the time of catheterization) that are involved in the consumption process. Our calculation of total consumption only approximates the extent of this process. This calculation may be an underestimate, since it does not include the consumption of newly formed unlabelled platelets, a number that we did not directly measure.

The calculated "effective production" reflects the marrow's ability to restore the peripheral platelet count to its normal level. It is unlikely that the presence of a catheter in the circulation could alter the activity of the marrow [which is chiefly under hormonal (thrombopoietin) control], and actual platelet production at least as likely in the experimental group as in the control group.⁷ Calculated "effective production" (net platelets plus consumed platelets) is underestimated to the same extent as is total platelet consumption (see above). That the "effective production" in the experimental group was less than that in the control group restates the phenomenon that consumption is of a greater magnitude than production. Eventually, a new steady state would arise whereby production and consumption would equalize at a lower peripheral platelet count. In all likelihood, the marrow would actually increase platelet production to compensate for the increased losses of platelets.

Many platelets taken out of the circulation appear at clot on the catheter. Swan-Ganz catheters are fabricated from polyvinylchloride, a known thrombogenic material.^{2,8,9} The clot formation seen along the catheter and pulmonary artery from one representative dog is similar to that found in man.² However, catheter-induced thrombocytopenia has not been described previously. Foreign surfaces have been shown to increase platelet adhesiveness, resulting in clot formation and predisposition to subsequent adhesion,

causing continued deposition of platelets with time.^{9,10} Foreign surface-induced platelet activation increases platelet phagocytosis by the reticuloendothelial system.⁴ This could account for the continuous decline in platelet count during catheterization. Such a mechanism remains unproved.

Despite species differences, human and dog platelets adhere similarly to polyvinylchloride.^{4,11-15} That catheter-induced thrombocytopenia occurs in man remains conjectural.

This study also emphasizes that interpretation of platelet kinetics in dogs subjected to pathologic states (e.g., shock) must take into account the abnormalities induced by catheterization.

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materials. For more than twenty years, this Company and the industry have initiated and supported efforts to assure the safe use of these products. These efforts have included research dealing with product safety, the publication of appropriate product literature, and an unprecedented level of cooperation with standard setting groups such as the National Sanitation Foundation. In keeping with our continuing efforts to evaluate the safety of our products and their uses, I am submitting the following comments on the Summary Report of Major Findings of California Pipe Trades Council Health Survey, July 1980. A/V

1. One of the major flaws in the survey is that there is no control (non-exposure) group. Without a control, a study is meaningless.
2. Another major weakness of this survey is that it lacks objectivity, particularly because it is not based on actual medical records.
3. The report does not identify the total cohort to which a questionnaire was sent. Therefore, it is impossible to determine what portion the 10,200 analyzed questionnaires represents.
4. The report states (page 4) that 6,508 responses were omitted because they did not complete the questionnaire. While it is understandable that complete cooperation cannot be expected, this does not mean that this group is not important to the study. The study also states (p. 4) that over 80% of non-respondents that were contacted did not respond because they no longer were working in the pipe trade. Again, this does not mean that their lack of inclusion in the study is not important; in fact, they are very important.
5. The report (page 5) defines the high and low exposure cases on the basis of duration of work. This use of the terms "high" and "low" are not appropriate since they denote high/low levels of unspecified agents and since duration of employment is not equivalent to level (high or low) of exposure. Secondly, there is no data available

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which indicates that exposure to "plastic" related chemicals are above levels that are harmful. In fact, studies reported by HALTS indicate that exposure to certain solvents used in solvent cements are well below the OSHA and ACCIH TLV's based on time-weighted averages. A more appropriate category is "long and short duration of employment".

6. The Union report (page 5) states that welders were excluded from this survey in order to eliminate another variable which might produce health effects. It is interesting that while the Union is concerned about potential health effects that might result from plastics use, it seems less concerned about potential health hazards to its members who also weld. Further, if the Union is interested in singling out "plastics", an objective study based on actual medical records should be conducted and should separate those people who are exposed to potential hazard from metal pipe use. Any study of this nature should identify and separate those who were involved with metal pipe use, and it should detail the kinds of pipe and what types of joints (e.g. soldering, brazing, pouring hot lead, gas welding, electric welding). It is difficult to find the merit, that the Union apparently sees, in identifying "plastics" as "the" hazard.
7. On page 5, it is stated that an effort to establish reliability and validity of the data was made by telephone interviews with a number of non-respondents. It is difficult to see how this addresses reliability and validity since it does not include any objective base such as medical records. Furthermore, the report does not state how many telephone interviews were conducted (i.e., were 6 eligible cases found out of 10 calls, 6 out of 100, etc.).
8. The report states that "trained telephone survey interviewers" were used. However, it does not indicate the nature of this training. This is important since the interviewer, in addition to the questionnaire, can bias the response.
9. On pages 6 and 7, the report suggests the use of this survey for a case controlled analysis. Because of the numerous flaws in this survey, there is no way that this study is now or will be of any use in studying potential occupationally-related health effects.
10. On page 10, the report implicates butadiene-styrene and PVC with induction of malignancies of the lymph system. It should be noted that the referenced NIOSH survey found an excessive number of leukemias but determined that they were not statistically different from the control population. Secondly, PVC has never been shown to cause cancer of the lymph system.
11. On page 13, the report states that many of the chemicals used in plastic pipe fabrication are proven or suspected carcinogens. This statement is false. It is not referenced nor can it be.
12. The results reported on pages 7 through 11 note many effects which are suggested to be due to chemicals used in plastic fabrication. Again, we emphasize that without a control, it is impossible to

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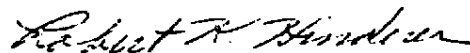
determine whether these are above the normal range. Also, the HALTS data does not indicate that exposure levels in the workplace are even close to a level which could produce an effect.

13. There is no attempt in this study to adjust the data for the effects of age, smoking, or drinking. Figure 1 is a prime example of this. Muscle weakness is a general symptom and can be caused by certain chemicals or by aging.
14. It is difficult to understand why the Union has used such a subjective approach when every suggested effect except sleepiness can be measured by common diagnostic techniques.
15. It is interesting to note that the report states that there is no effect of smoking noted. Since the effects of smoking are so dramatic, every study shows this effect. The absence of any smoking effect further serves to emphasize the inability of such a survey to identify major or minor compound-related effects and clearly shows that the survey is suspect as a tool in the investigation of occupationally-related diseases.

I believe that properly conducted epidemiology studies can play an important role in providing a low-risk environment. However, inadequate and ill-conceived studies such as this one do not serve the interest of either the public or the workers. In fact, such surveys are more inclined to disservice, since they can unduly alarm the workers and the public, erroneously direct research efforts, and can potentially result in unwarranted regulation causing unnecessary expenditure of resources.

Sincerely,

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