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**SUMMARY TECHNICAL REPORT
SNPO AND SNAP BIOLOGICAL STUDIES**

R. C. THOMPSON

JANUARY 3, 1966

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By

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Biology Department

Sponsored by:

U.S. Naval Radiological Defense Laboratory
San Francisco, California

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INTRODUCTION

These studies were undertaken pursuant to a requisition (cross service order) No. N62479/5034-0450 forwarded via the Richland Operations Office of the U.S. Atomic Energy Commission. This requisition, dated 6 April 1965, covered a work period of 6 mo and required submission of a summary technical report.

A sharp distinction can not be made between those phases of our investigations which were primarily concerned with SNPO (Space Nuclear Propulsion Office) problems and those phases concerned with SNAP (Systems for Nuclear Auxiliary Power) problems because the objectives in either case were very similar. Therefore, we are not separating this report into SNPO and SNAP sections. However, if such distinction is necessary, the ^{238}Pu feeding study is primarily related to SNAP and the studies with the Oak Ridge-prepared simulated fuel particles are primarily related to SNPO. The "large particle" inhalation studies and the solubility studies clearly have application to both SNPO and SNAP problems.

Our experimental work has consisted of several separate investigations which are described below. In many instances, these investigations are still continuing and final conclusions cannot be drawn. In addition to the experimental work described, considerable efforts were expended in the preparation and review of reports of the SNPO Radiological Effects Working Group.

Investigators involved with the specific problem areas are identified; overall direction of this effort was supplied by Dr. Roy C. Thompson.

"Large-Particle" Inhalation Studies

Investigators: Dr. B. O. Stuart
Dr. W. J. Bair
Dr. W. J. Clarke

Prior studies in "large particle" inhalation have been usually concerned with particles in the size range less than 5 μ . This was due to the generally held opinion that larger particles would not reach the alveolar lung and would be rapidly and completely cleared from the upper respiratory tract. Work with larger particles also involves rather severe problems of suspension and sampling. These larger particles, however, are of interest to SNPO and SNAP hazard evaluation because they are predicted to occur in significant quantities, and because only these larger particles are apt to be sufficiently radioactive to constitute a significant hazard.

Studies were performed in four beagle dogs exposed to aerosols of ceramic microspheres labeled with ^{59}Fe (obtained from Minnesota Mining and Manufacturing Company). Two of the dogs were exposed to particles of 10 μ , nominal diameter, and the other two dogs were exposed to particles of 20 μ , nominal diameter. Particle size analysis of the 20 μ material showed a count median diameter of 26 μ , with about 1% of the particles ranging below 16 μ ; the smallest size observed was 6.2 μ . Considerable difficulty was encountered in obtaining uniform dispersion of these large particles. Specially designed exposure chambers were developed which proved adequate for these particles but unsatisfactory in attempts to disperse 50 μ particles.

The difficulties encountered in these preliminary experiments prevented an accurate measurement of the total quantity of material inhaled. The fraction of inhaled material which was initially deposited can therefore only be roughly estimated at between 2 and 10%. Retention of this initially deposited material was followed by whole-body counting, by analysis of excreta, and by analysis of

tissues upon sacrifice of one animal from each group at 2 mo post-exposure. One-half to three-fourths of the initially deposited material was cleared within a few days postexposure; thereafter excretion was quite negligible. Twenty to 30% of the initially deposited 10 μ particles and from 30 to 60% of the 20 μ particles were still retained 2 mo after inhalation.

Whole-body scanning was accomplished by passing the animal through an array of columnated counters. For both 10 μ and 20 μ particles, the majority of the activity, immediately postexposure, was localized in the area of the lung with smaller amounts extending from the naso-pharynx throughout the tracheal region. By 4 hr postexposure, activity in the head and throat regions had largely disappeared; but a spread of the "lung peak" over the abdominal area indicated movement of swallowed material through the gastro-intestinal tract. By 24 hr postexposure, the "abdominal spread" had disappeared and activity was sharply peaked in the lung area where it remained, essentially undiminished, for 2 mo.

Of the total activity collected in the excreta, 70 to 80% was eliminated with the first day's feces; representing, without doubt, material removed from the respiratory tract by ciliary escalation and swallowed. The rate of ^{59}Fe elimination via the feces decreased more than a hundred-fold during the first 10 days postexposure. Elimination of ^{59}Fe in the urine was initially a hundred-fold lower than elimination in the feces, and may well have been even lower since some contamination with feces may have occurred in collecting the urine. The rate of elimination in the urine dropped less rapidly than that in the feces and during the period from 10 days to 2 mo postexposure about half as much activity appeared in the urine as in the feces. During this period the total daily excretion amounted to no more than a few tenths of a percent of the activity remaining in the lung. Because of the increasingly significant role of urinary excretion with the time postexposure it seems likely that ^{59}Fe excreted after the first few days represents

that which was leached from the deposited particles rather than removal of the particles themselves.

Analyses of tissues taken from two animals sacrificed 2 mo after inhalation of the particles showed that practically all of the ^{59}Fe was confined to the lungs. Multiple transverse sections (20 μ thick) were taken of the various lobes of the lung to locate and identify particles by autoradiography. Twenty particles were observed in 64 microscopic sections from the dog that had inhaled 20 μ microspheres, and 65 particles were observed in 78 sections from the dog that had inhaled 10 μ microspheres. There was a tendency, particularly apparent with the 20 μ particles, for preferential deposition in the right lung. In most instances, particles were located in the deep lung, adjacent to alveolar walls. There was also evidence on the autoradiograms of ionic ^{59}Fe , no doubt representing activity leached from the microspheres.

Conclusions: These were preliminary experiments involving only four dogs, two of which are still under observation. Quantitative conclusions are clearly not justified. However, certain very significant qualitative observations were made. Particles as large as 30 μ in diameter are respirable, as evidenced by direct measurement in tissue sections. These particles penetrate to the alveoli and are tenaciously retained. Twenty to 60% of initially deposited material in the size range of 5 to 30 μ was retained for 2 mo postexposure, and the very slow loss of activity after the first few days seemed due to leaching of ^{59}Fe from the particles rather than removal of intact particles. More work is necessary if inferences are to be drawn as to the fraction of inhaled material initially deposited, and if the effect of particle size, shape, and density on deposition and retention kinetics is to be understood. Future studies should also be planned to investigate the relative biological consequences of exposure of the lung to radiation from a few large particles as compared to the effect of the same radiation dose more generally distributed.

Miniature Swine Intragastric and Skin Exposure Studies

Investigators: Dr. J. R. McKenney
Dr. R. O. Shannon
Dr. H. A. Ragan

These studies employed simulated reactor fuel particles obtained from the Health Physics Division, Oak Ridge National Laboratory. These particles were about 1 mm in diameter and consisted of depleted uranium in a graphite matrix. They were spiked with ^{89}Sr (added as chloride). Each source contained about 700 μCi ^{89}Sr plus 40 μCi ^{90}Sr - ^{90}Y . Dose rates were 13,000 rad/hr at the surface and 4000 rad/hr at a tissue depth of 50 mg/cm^2 .

Gastric mucosal exposures were made using intragastric balloons to which six of the sources were cemented. Fasted swine were anesthetized, positioned on their backs in a trough, and a deflated balloon inserted through the mouth into the stomach. The balloon was then inflated with water to insure contact between the sources and the gastric mucosa. Simultaneous skin exposures were performed with one source on each side of the animal on the lateral cutaneous surface of the abdominal wall. Exposures were either 1 or 4 hr in duration.

A patchy congestion of the mucosa was the only gross change observed. This appeared in three swine sacrificed 2, 3, and 15 days following the 1 hr exposure and in two swine sacrificed 1 and 3 days following the 4 hr exposure. Since the precise location and proximity of the sources to the gastric mucosa could not be determined, correlation of the observed congestion with the position of the source was not possible. Focal lesions were not observed. The congestion was absent 5 wk following the 4 hr exposure. No congestion was observed 2 days following 1 hr exposure of a control animal to a balloon without attached sources.

Skin effects were more pronounced. A slight localized erythema developed 15 to 20 days after the 1 hr exposure. A slight erythema

was observed immediately following the 4 hr exposure; a more severe erythema developed after 2 to 3 wk, followed by necrosis of an area about 3 mm in diameter; and a crusted ulcer appeared after about 4 wk. Healing of the lesions was slow, being incomplete after 10 wk.

Conclusions: These are very limited and preliminary observations. The greater severity of the skin lesions suggest that early reaction of the gastric mucosa to particulate radionuclide sources would be less severe than that of the better understood and more easily studied effects on the skin. However, the gastric mucosal exposures were less well controlled than the skin exposures. Displacement of the sources along the mucosal surface could well account for the less severe and more diffuse reaction. The experimental limitations of the balloon technique suggest that alternative approaches to this problem might offer greater promise than efforts to refine and extend the present studies.

Ingested Particle Studies in Rats

Investigators: Dr. M. F. Sullivan
Dr. T. J. Mahoney

The same simulated fuel particles 1 mm diameter, ^{89}Sr spiked, described in the previous section were employed in this study with rats. These particles were administered by intragastric intubation, singly or 6 to 10 particles at a time, to individual rats. The particles were recovered from the feces and repeatedly reused. Studies were made of passage time, radiation dose to various segments of the gastrointestinal tract, and gross and histological damage. The particles employed proved rather unsatisfactory for certain purposes of the experiments because of the pronounced tendency for leaching of the ^{89}Sr . Thus, in the first passage of these particles through a rat, the measured surface dose was decreased by about 50%. The rate of leaching was substantially reduced in subsequent passages. This leaching seriously complicated the dosimetry problem but did not affect measurements of passage time or certain qualitative conclusions regarding histological effects.

Of 215 particles administered intragastrically and recovered from the feces, 37 were excreted during the first day, 118 on day 2, 41 on day 3, 11 on day 4, and 8 on days 5 through 8. Since the normal passage time for the rat is of the order of 15 to 24 hr it is apparent that these large particles do show a tendency to be held up in their passage through the tract. The radioactivity of the particles fed on different occasions varied widely due to leaching and radioactive decay of the ^{89}Sr . There was no indication that prolonged retention of a particle was associated with a high radiation level.

In most of these experiments glass dosimeters were surgically implanted along the gastrointestinal tract. Interpretation of the measured radiation doses was complicated by the knowledge that extensive leaching of ^{89}Sr was occurring. Higher than average

doses were most frequently observed in the terminal colon and less frequently in the cecum and stomach. Gross symptoms of gastrointestinal radiation effects were usually observed only when glass dosimeter readings of as much as 500 rads were measured.

Histopathologic examination provided definite evidence of focal damage attributable to lodged particles. Of four animals fed single particles, three exhibited such focal lesions in the stomach, cecum, and colon despite the fact that only a small fraction of the total tract was sectioned and examined. Lesions occurred more frequently in animals to whom several particles were administered. These lesions were sharply localized and on cross section did not occupy more than one-third of the mucosal circumference. Maximal involvement was seen in the center of the lesion with a degree of damage equivalent to that produced by 1500 R external X-ray. In areas away from these lesions, the mucosa appeared normal. Damaged areas were most frequently observed in the dependent glandular stomach, the lower ileum, the cecum, and the colon.

The relatively high incidence of the focal lesions was surprising since one might have expected that the particles would usually be imbedded within, and shielded by, the intestinal contents. Examination of the excreted fecal pellets containing ⁸⁹Sr labelled particles disclosed that in nearly every instance these particles were located at the surface of the pellet.

Conclusions: Although performed in the rat, these experiments may be qualitatively applicable to man. They suggest that holdup of particles in the intestinal tract may pose significant problems, i.e., that the usual assumptions regarding passage time may not be applicable to particles. Of perhaps greatest significance is the indication that the position of the particle within the lumen of the intestine is not determined by chance, but that it may be preferentially located against the wall of the intestine. Thus

it may be necessary to develop new models for both rate of passage and dosimetry calculations for particles traversing the gastrointestinal tract. The effect of particle size, shape, and density need to be further elucidated. Such information may best be surveyed in small animals, but findings will need to be checked in large animals, and, to the extent possible, in man. Observation of the degree and incidence of focal damage appears to be the most direct approach to the evaluation of detailed particle movement through the tract. The use of glass dosimeters implanted along the gastrointestinal tract of a large animal, such as the miniature swine; together with nonleaching radionuclide-labeled particles, should provide pertinent information.

Plutonium-238 SNAP Fuel Ingestion by Miniature Swine

Investigators: Dr. V. H. Smith
Dr. J. L. Palotay
Dr. B. J. McClanahan
Dr. H. A. Ragan
Dr. W. J. Clarke

A single pig was fed a massive dose (approximately 1 Ci) of ^{238}Pu SNAP fuel particles. This conceptually simple experiment proved to be quite a major undertaking because of the very large quantity of highly radiotoxic material involved, and because of the marked propensity of this material to become airborne during any handling operation. In the procedure which finally evolved, the PuO_2 was added through a brass funnel (to avoid sticking) to a No. 4 gelatin capsule containing CaCO_3 . The funnel was rinsed into the capsule with CaCO_3 , the capsule sealed, and placed in a No. 1 gelatin capsule containing NaHCO_3 . This capsule complex dissolved and released the contents after 6 to 8 min exposure to simulated gastric juice at 37 °C. Irradiation with 50,000 $\text{R } ^{60}\text{Co}$ gamma rays had no effect on capsule fragility or solubility. The CaCO_3 in the inner capsule kept most of the PuO_2 from contact with the gelatin walls; the NaHCO_3 was used in the outer capsule for insulation. The capsule was "sealed" into the catheter with a sugar-glycerol mixture (which does not harden, nor dissolve the capsule) and after insertion of the catheter, pushed out into the pig's stomach.

The SNAP fuel particles employed were standard production material obtained from Mound Laboratory. The pig was a 49.2 kg female Hanford Miniature, which had been acclimated to the metabolism cage for 3 days and was eating normally. Total urine and feces collections and periodic blood samples were obtained for 14 days following PuO_2 feeding. The pig was then sacrificed and tissues analyzed. Feces were gamma-counted; all other samples were alpha-counted after treatment according to standard TTA extraction techniques. Results of these analyses are shown in Tables I and II.

Table I. ^{238}Pu Content of Feces, Urine, and Blood

<u>Day</u>	<u>Feces^a (mCi)</u>	<u>Urine (pCi)</u>	<u>Blood^b (nCi)</u>
1	no feces	118	78
2	144	10	83
3	69	7	293
4	168	144	25
5	54	292	
6	44	16	10
7	125	20	
8	192	4	6
9	151	6	
10	44	14	4
11	4	13	
12	2	9	
13	2	4	
14	2	8	0.0

^a Normalized to a total 14 day excretion of 1 Ci.

^b Assuming 8% of body weight is blood.

Table II. ^{238}Pu Content of Tissues at 14 Days Postingestion

<u>Tissue</u>	<u>Content (nCi)</u>	<u>Concentration (pCi/g wet weight)</u>
Lungs	0.08	0.34
Spleen	0.09	1.2
Muscle ^a	1.5	0.71
Kidneys	0.10	1.0
Heart	0.08	0.56
Liver	0.31	0.43
Skeleton	0.25	0.12
Lymph Nodes		
Gastric	114.	
Mesentric	0.37	
L. suprainguinal	13.	
Aortic	0.10	
Bronchial	4.4	
L. prefemoral	8.4	
L. prescapular	22.	
Ext. iliac	3.0	

^a Assuming 42% of body weight is muscle.

It is apparent that there was prolonged and extensive hold-up of the particles in the intestinal tract. Slightly less than half of the ingested activity was excreted during the first 6 days. The remaining activity was, however, almost totally excreted during the next 5 days. At autopsy there was no evidence of hot spots in the intestines, which were carefully surveyed with a thin-window counter sensitive to ^{238}Pu X-rays. The somewhat bimodal pattern of ^{238}Pu excretion in the feces is intriguing. A relationship between the period of holdup and particle size is suggested. Efforts will be made to study the distribution of particle sizes in each days fecal collection.

The total ^{238}Pu recovered in the urine and in the tissues at sacrifice amounted to 2×10^{-7} times the amount fed. Over 98% of this absorbed plutonium was recovered from lymph nodes. Such predominant deposition of plutonium in lymph nodes is not a typical observation. Plutonium reaching the blood stream is deposited predominantly in liver and bone. Therefore, the lymph node plutonium most likely was absorbed in particulate form via the lymphatic system. If one disregards the ^{238}Pu in lymph nodes, then only about 3×10^{-9} times the amount of ^{238}Pu fed was truly solubilized and absorbed from the gastrointestinal tract into the blood stream.

The ^{238}Pu deposited in lymph nodes may represent a small fraction of dust accompanying larger particles. This dust may, in a sense, be looked upon as the most hazardous component of the mixture, because it may be presumed to account for 98% of the internally deposited plutonium resulting from this ingestion. From other standpoints, however, it cannot be considered a really significant hazard because of the very small fraction deposited (2×10^{-7}) and because lymph nodes may be a relatively safe place to deposit plutonium. In other studies which we have performed, inhaled plutonium deposited in the pulmonary lymph nodes of dogs has not been observed to translocate to other tissues and has not resulted in other than local damaging effects.

Despite the very large dose of $^{238}\text{PuO}_2$ administered and the relatively long holdup of the material in the gastrointestinal tract, there was no gross pathology evident on close inspection of the tract at autopsy. Histological sections were taken but have not yet been examined.

Conclusions: The results from one experiment in one pig can scarcely be considered definitive for man. Confirmatory studies are clearly desirable. However, the results from this one pig were so unequivocal as to leave little doubt as to the negligible ingestion hazard from this material. The relatively enormous dose of 1 Ci resulted in no grossly evident damage to the gastrointestinal tract, and to negligible deposition in the probably most critical organs, bone, and liver. Any conceivable hazard from ingestion of this material would certainly be far outweighed by the accompanying hazard of inhalation.

Solubility Kinetics of Particles

Investigator: Dr. V. H. Smith

Some very preliminary studies have been initiated in this area. We have developed what appears to be a reasonable simulant for human gastric juice. The composition of this solution is shown in Table III. This formulation is being employed at USNRDL in studies of the solubility of SNPO fuel element materials.

Table III. Composition of Simulated Gastric Juice

Gastric mucin	360 mg
Pepsin (3x crystallized)	1 mg (~ 3200 Hb units)
Ascorbid acid	1 mg
Urea	3 mg
Uric acid	1 mg
KCl	150 mg
CaCl ₂ ·6H ₂ O	40 mg
NaCl	280 mg
Sialic acid (25.5% assay)	29.2 mg (7.4 mg sialic acid)
Sodium glucuronate	2.2 mg
HCl	7.8 meq (to give pH of 1.0)
H ₂ O	to total volume of 100 ml

We are working on a method of solubility measurement which we hope will be applicable, in a standardized fashion, to a wide variety of particles and fluids. The method, as presently visualized, will deal with small numbers of particles handled on an essentially individual basis without intervention of membranes or filters. A cylindrical thermostat controlled chamber will be employed and will have a flat glass bottom through which the particles may be observed and photographed for measurement of particle size and shape throughout the period of equilibration. No systematic application of this technique has as yet been attempted.

General Conclusions

The experimental results described in this report are mostly quite preliminary in nature. They provide no final answers to SNPO and SNAP hazard evaluation problems, but they do suggest some qualitative conclusions and demonstrate the means by which more definitive answers may be sought.

Particles in the 5 to 30 μ size range are respirable (by dogs) and cannot be ignored in evaluations of inhalation hazards. The 100 to 1000 μ particles may be held up in the gastrointestinal tract (of pigs and rats) for periods of many days. Particles within the contents of the lumen of the gastrointestinal tract (of rats) cannot be assumed to be randomly distributed. In a specific instance, the internal deposition of ^{238}Pu (in a pig) following ingestion of a massive dose of $^{238}\text{PuO}_2$ SNAP fuel particles was quite insignificant. There was an indication that the most hazardous aspect of these particles may be the small amount of very fine "dust" associated with them. A quantitative assessment of the hazard implications of all these various observations in terms of their dependence on such factors as particle size, shape, composition, and density will require much more extensive investigation.

Also relatively untouched in these preliminary studies was the problem of biological effects to be anticipated from particulate radiation sources. Such effects presently must be predicted from our more extensive knowledge of the effects of more generalized radiation exposure. Little confidence, however, can be attached to such predictions, and comparative studies of the effect of point source versus extended source irradiation are badly needed. This need exists specifically for SNPO and SNAP problems of skin, lung, and gastrointestinal tract irradiation. Both short term and long term effects should be investigated.

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