ELSEVIER

The Science of the Total Environment 274 (2001) 161–169

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

www.elsevier.com/locate/scitotenv

Pet dogs as sentinels for environmental contamination

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Received 20 May 2000; accepted 2 August 2000

Abstract

The presence of environmental contaminants in air, water and food may pose significant health risks to the exposed human population. However, problems associated with assessing chronic exposure to low doses of environmental chemicals, multiple exposure routes, diseases with long latency periods, and non-specific health outcomes make it difficult to conduct the appropriate human epidemiologic studies. It may be useful to complement human epidemiology with animal studies. Animals monitored or evaluated in situ for the appropriate suite of endpoints can provide information about both exposure levels and potential adverse health effects. Animals have served as sentinel indicators for health effects associated with a number of environmental exposures, including pesticides and asbestos. Pet dogs may be particularly valuable sentinels because they share the human environment. In addition, dogs respond to many toxic insults in ways analogous to humans, they have physiologically compressed life spans, and they are free from some important lifestyle risk factors for disease. An example of how pet dogs may be used as sentinels for potential human health hazards involves a study of the genotoxic effects resulting from exposure to a mixture of chemicals from nearby Superfund sites. We conducted a cross-sectional study of exposed dogs (living in the community with the Superfund sites) and controls (living in a nearby community). The pet owners completed a questionnaire, and we collected a blood sample from each dog. The blood samples were analyzed for standard clinical parameters and assays for possible genotoxic effects (peripheral blood lymphocyte micronucleus frequency and lymphocyte subtyping). Pet dogs living near the Superfund sites had a higher micronucleus frequency than control animals, suggesting that the dogs may have been exposed to environmental contaminants from these sites.

Keywords: Bioassays; Environmental contamination; Biomarkers; Sentinel animals; Dogs; Peripheral blood lymphocyte micronucleus; Lymphocyte subtyping

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0048-9697/01/$ - see front matter
PII: S0048-9697(01)00740-9

1. Introduction

The presence of environmental contaminants in air, water and food may pose significant health risks to the exposed human population. However, chronic low-dose exposure, multiple exposure routes, long latency periods, and non-specific health outcomes make it difficult to conduct the appropriate human studies needed to demonstrate causality. It may be useful to complement human epidemiologic studies with similar studies conducted on sentinel species, such as companion animals (e.g. pet dogs, cats, birds, horses). Animals monitored or evaluated in situ for the appropriate suite of biomarkers can provide information about both exposure levels and potential adverse health effects. The following is a brief literature review, as well as a specific example, of how pet dogs have served as sentinels for human health effects resulting from exposure to environmental contaminants.

1.1. Animals as sentinels

The concept of using animals as sentinels for adverse human health outcomes is not new. Probably the most widely recognized animal sentinel is the canary used in mining exploration to detect carbon monoxide. The idea of the 'canary in the coal mine' has been expanded to take advantage of resources such as in situ animal monitoring, wildlife mortality data, and animal tumor registries to monitor for environmental health hazards [see National Research Council (NRC, 1991) for review; van der Schalie et al., 1999].

In addition to using available resources and data bases to identify environmental health hazards, it may also be useful to conduct epidemiologic studies using the appropriate sentinel animal populations. Descriptive epidemiologic studies can be used to estimate disease frequencies and geographic distributions. The observation of disease clusters may suggest a common environmental etiology. Analytic epidemiologic studies can be used to test hypotheses regarding environmental exposures. In situ studies of wild animal populations or of animals housed on a particular site can be used to assess bioaccumulation as well as ecological or health effects.

Companion animals can be particularly valuable sentinels for human exposures because they share much of their environment (e.g. air, water, food) with people. Pets may be even more exposed than their owners to some contaminants, such as soil or house dust. Animals respond to many toxic insults in ways analogous to humans, and they can develop similar environmentally induced diseases by the same pathogenic mechanisms. Because animals typically have shorter, physiologically compressed life-spans when compared with people, latency periods for the development of some diseases are shorter in animals. Also, animal studies are free from some of the confounders (lifestyle and occupational risk factors) that can make the results of human studies difficult to interpret. Finally, as in humans, endpoints measured in biological samples from animals represent an integrated measure of exposure to ambient environmental contamination (see NRC, 1991).

Evaluating or monitoring the appropriate biological markers in companion animals, particularly pet dogs, could reduce some of the uncertainty associated with predicting human risk. It may be easier to couple measures of environmental exposure with clinical or subclinical effects, allowing the detection of biological effects that precede overt disease.

Pet dogs have tremendous potential as sentinels for environmental health. This has been demonstrated in a number of studies with known environmental carcinogens. For example, a study of pet dogs with mesotheliomas identified household asbestos exposure (e.g. from asbestos-related hobbies, such as auto mechanics; the use of flea powders containing talc; or clothing contaminated by occupational activities) as risk factors that might also increase the human risk of asbestos-related disease (Glickman et al., 1983). Hayes et al. (1981) reported a dose-dependent association between household applications of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and malignant lymphomas in dogs. These findings were consistent with reports of a modest association between agricultural exposure to 2,4-D and an

increased risk of non-Hodgkin's lymphoma in people (Hoar et al., 1986). Reif et al. (1992) conducted a case-control study of canine lung cancer and found, among short-muzzled dogs, an increased risk of lung cancer from exposure to environmental tobacco smoke.

In addition to manifesting similar responses to specific environmental carcinogens, dogs and people may suffer similar illnesses when exposed to the same general environmental conditions. For example, studies have shown that living in an urban environment increases the risk of canine pulmonary disease (Reif et al., 1970) and bladder cancer (Hayes et al., 1981). The geographical distribution of canine bladder cancers was similar to that of human bladder cancers (Hayes et al., 1981).

Other studies have shown that pet dogs can be good predictors of environmental exposure in people. For example, Ostrowski (1990) demonstrated that dogs can serve as predictors of blood lead levels in children exposed to a lead-contaminated environment.

In summary, a number of studies have shown that pet dogs respond to at least some environmental contaminants, such as asbestos, herbicides, and in an urban environment, respond similarly to the way humans respond. Pet dogs have also been found to be good predictors of children's exposure to lead. Given that dogs and people can suffer similar illnesses when subjected to the same risk factors, it is likely that monitoring the health of pet dogs will help identify associations between exposure to environmental contaminants and the occurrence of disease. Below is a discussion of a study examining whether pet dogs could serve as sentinels for human exposure to and biological effects from environmental contamination from two hazardous waste sites.

2. Pet dogs as sentinels for environmental exposures from two Superfund sites

The study area was a small town of 2700 people in North Carolina that had two Superfund sites [see US Environmental Protection Agency (EPA, 1990)]. Six sites located within the Superfund study area were contaminated with high levels of organochlorine pesticides, including DDE, DDT, and lindane; as well as volatile organic chemicals. Soil (including airborne dust), ground water (the source of drinking water), surface waters (including a recreational lake), and fish have been contaminated by runoff and seepage from the sites (EPA, 1990). Private and city wells have been closed, primarily because of the presence of lindane [see US Department of Health and Human Services (DHHS, 1992)].

In 1985 and 1986, the EPA initiated remediation activities at some of these sites. Following remediation, the levels of pesticides in the soil were below EPA standards of 50 parts per million (ppm); however, pesticides, including aldrin, DDD, DDT, heptachlor, and toxaphene were still present in the soil (Gill et al., 1991).

In addition to the environmental sampling done by the EPA, biomonitoring studies were conducted at the sites using the *Tradescantia* micronucleus assay (Gill et al., 1991). Prior to remediation, the mean frequency of micronucleated tetrads for plants grown onsite in contaminated soil (five soil plots) ranged from 9.3 ± 0.53 (standard deviation, S.D.) to 17.9 ± 4.65, compared with 7.1 ± 0.52 for laboratory-grown control plants. Even after remediation, plants grown onsite on one of four plots had higher frequencies of micronucleated tetrads (7.7 ± 0.66) than laboratory-grown control plants (6.8 ± 0.45).

The results from the plant assays demonstrated that the soil and air concentrations of contaminants at the site were still high enough to induce genotoxic effects. In 1992, the area was categorized as being a continuing public health concern because of the risk to human health caused by exposure to widespread contamination at the sites, which were easily accessible by the public, and by the ingestion of contaminated ground water (DHHS, 1992). We were interested in determining whether pet dogs, who would be exposed to the contaminated tap water as well as airborne contaminants, would be good sentinels for human exposure.

2.1. Selection of biomarkers

If dogs are to serve as sentinel animals for adverse health effects in both animals and people, a biomarker or set of biomarkers likely to be affected by the suspected exposures must be examined in the pet population. Some of the chemicals present at these sites interact with DNA, and exposure to DNA-damaging agents can produce acentric chromosome fragments and chromosomes with disrupted spindles, both of which can be detected in the cytoplasm as micronuclei (Heddle et al., 1983). For our study, we examined the frequency of micronuclei in cultured peripheral blood lymphocytes (PBLs).

Exposure to environmental contaminants can also induce adverse effects on the immune system (Vos, 1977, 1986). For example, Fiore et al., (1986) found a lower T4 +/T8 + lymphocyte ratio (ratio of T-cell lymphocyte subsets) in women exposed to groundwater contaminated with the pesticide aldicarb than in a control group of women. In our study, we determined CD4 +/CD8 + lymphocyte ratios in dogs. We also analyzed blood samples for complete blood count (CBC) and the standard serum chemistry profile.

2.2. Study design and recruitment

We conducted a cross-sectional study involving exposed and unexposed dogs. Exposed dogs were recruited from the town with the Superfund sites. Maps of the community that identified the location of the Superfund sites indicated that all residents in the town lived within 1.5 miles of one or more sites. For our study, animals living within this area were defined as exposed and were eligible for inclusion.

Control animals were selected from a nearby town matched for a similar population size and similar geographic location. Sources of drinking water in the control town were also similar (i.e. public and private wells that tap an underground aquifer). None of the wells serving the control town were contaminated with pesticides, and no Superfund sites were known to be located within the control town boundaries.

Local veterinarians cooperated in recruiting study subjects. The owners of all dogs visiting these veterinarians for annual routine care during the period between June 1992 and September 1992 and meeting study criteria (clinically healthy [negative stool sample, negative result on a heartworm test, no overt disease (i.e. clinically normal)], at least 5 years old, and living in the study or control area for at least the last 4 years) were asked to participate. Participants were asked to complete a questionnaire, and a 15-ml blood sample was obtained from each dog in the study.

2.3. Biomarker assays

We cultured peripheral blood lymphocytes (PBLs) as described in Erexson and Kligerman (1987), but with minor modifications in culture times: 3 μg/ml cytochalasin B (Sigma Chemical Co, St Louis, MO) was added to each culture at 48 h, and we harvested the cultures after 96 h. We analyzed 1000 binucleated PBLs per dog for micronuclei. In addition, we determined nucleation indices, (i.e. the percentages of mono-, bi-, tri-, and quadrinucleated cells) by counting 200 consecutive cells per dog.

We conducted flow cytometry analyses according to the procedure described by Gebhard and Carter (1992) using a FACScan equipped with Consort-30 Software (Becton–Dickenson Immunocytometry Systems, Sunnyvale, CA). Lymphoctyes were incubated with fluorescein-conjugated goat anti-mouse IgG (Organon-Technika, West Chester, PA) and then with streptavidinphycoerythrin (Serotek, Kidlinton, UK). For each sample, we collected 15 000 cells, and performed gating on the forward angle scatter, side scatter, and two-color fluorescence.

We performed serum chemistry analyses (albumin, alkaline phosphatase, alanine aminotransferase, total bilirubin, blood urea nitrogen, calcium, creatinine, glucose, phosphorous, total protein, amylase, sodium, potassium, chloride, total CO_2, anion gap) on a Monarch 2000 (Instrumentation Laboratory, Lexington, MA). CBCs were performed using a Baker 9000 Biochem Immunosystem (Serono-Baker, Allentown, PA) and standard methodologies. The CBC (including hematology) parameters analyzed were: white

blood cell count, red blood cell count, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean cell hemoglobin concentration, platelet count, plasma solids, mean platelet volume, leukocytes, segmented neutrophils, band cells, eosinophils, basophils, lymphocytes, and monocytes.

2.4. Questionnaire

All of the people asked to participate in this environmental health study agreed to be included. Dog owners completed a questionnaire at the time the blood sample was taken. The questionnaire included questions regarding the dogs' age, breed, health history, exposure to flea and tick pesticides, diet, drinking water source, and exposure to second-hand smoke and other hazardous substances (e.g. from owners' hobbies, household pesticide use).

2.5. Statistical analyses

We performed statistical analyses using SAS (SAS, 1990). We computed descriptive statistics for the exposed and control groups for each bioassay endpoint and for the results of the questionnaire. We performed *t*-tests to examine whether the population of exposed dogs differed from the controls.

We calculated measures of the effect of environmental exposure on biomarker values as the exposed dogs' relative risks for having biomarker values outside the range of normal. We based determinations of normal/abnormal results on established parameters at North Carolina State University College of Veterinary Medicine Clinical Pathology Laboratory (from 75 dogs). There are no historical normal ranges established for either the PBL micronucleus assay or for CD4 + /CD8 + lymphocyte ratios in dogs. We created a 'normal' range using data from the control group in two different ways: (1) the range of values (PBL micronucleus frequency or CD4 +/CD8 + ratio) between the mean $-1 \times$ S.D. and the mean $+1 \times$ S.D.; and (2) the range of values between the mean $-2 \times$ S.D. and the mean $+2 \times$ S.D.

3. Results and discussion

We collected data for 20 exposed dogs and 21 controls. The primary difference between the households of exposed dogs and those of the control dogs was in the percentage of households getting their water from community water sources (two-thirds of the exposed group compared with one-half of the control group). The ages of the dogs in the two groups were essentially the same (i.e. 8.0 ± 2.53 years for the control group and 8.2 ± 2.74 years for the exposed group). The prevalences of other factors, such as the presence of smokers, a history of insecticides being used on the dogs, and a history of insecticide use in the homes, were also similar for the exposed and control households.

Results from the CBCs and serum chemistry panels for all dogs in the study were within the normal range as established by the North Carolina State University College of Veterinary Medicine (data not shown). There were no statistically significant differences in the mean values for these biomarkers between exposed and control dogs. However, the values for serum levels of alkaline phosphatase and alanine aminotransferase were more variable in the exposed group than in the control group (see Table 1).

Table 1
Results from the serum chemistry panel for the control and exposed dogs[a]

Parameter (IU/l)	Controls ($n = 21$)	Exposed ($n = 20$)
Alkaline phosphatase	46.3 ± 37.5	101.5 ± 150.0
Alanine aminotransferase	30.6 ± 17.8	38.6 ± 57.3

[a] Values are mean $\pm$ S.D.

Table 2
Results from the peripheral blood lymphocyte micronucleus assay for the control and exposed dogs[a]

Micronucleus endpoint	Controls ($n = 21$)	Exposed ($n = 20$)
Number of micronuclei per 1000 binucleates	11.0 ± 3.29	24.2 ± 10.39[b]
Percent micronucleated binucleates	1.1 ± 0.31	2.2 ± 0.73[c]
Nucleation index[d]	1.67 ± 0.21	1.68 ± 0.23

[a] Values are mean ± S.D.
[b] Significantly different from controls ($P = 0.0001$).
[c] Significantly different from controls ($P < 0.001$).
[d] The nucleation index is calculated as follows: (1 × frequency of mononucleate lymphocytes) + (2 × frequency of binucleate lymphocytes) + (3 × frequency of trinucleate lymphocytes) + (4 × frequency of quadrinucleate lymphocytes).

Results from the PBL micronucleus assay are presented in Table 2. Using the Satterthwaite method (Steel and Torrie, 1980) for a *t*-test using samples of unequal variances, we found that the exposed dogs had significantly more micronuclei ($P = 0.0001$) and a higher frequency of micronucleated binucleates ($P = < 0.001$) than the control dogs. There was no difference in the nucleation index for the two groups, indicating that, under similar culture conditions, the PBLs from both groups divided at the same rate.

The results from the PBL subtyping based on flow cytometry are listed in Table 3. The absolute numbers of CD4 + (obtained by using monoclo-

Table 3
Results from the immunophenotyping analysis using flow cytometry for the control and exposed dogs[a]

Cells labeled by monoclonal antibodies[b]	Controls ($n = 21$)	Exposed ($n = 20$)
CD4 + (clone 8.53.4)	432 ± 274	520 ± 298
CD8 + (clone 4.78.6)	299 ± 227	464 ± 324
CD4 + /CD8 + ratio	2.02 ± 1.2	1.50 ± 1.0

[a] Values are mean ± S.D.
[b] Per microliter of whole blood.

Table 4
Chi-square value estimates and the relative risk for abnormal values of biomarkers in the exposed group of dogs compared with the controls

Biomarker	Relative risk (95% CI[a])	Chi square (probability)
Elevated alkaline phosphatase level[b]	3.2 (0.36–27.8)	1.22 (0.27)
Elevated alanine aminotransferase level[c]	1.0 (0.24–4.61)	0.004 (0.95)
Elevated percent micronucleated binucleates[d]	7.9 (2.06–30.2)	18.1 (0.000)
Elevated percent micronucleated binucleates[e]	14.7 (2.1–101)	18.8 (0.000)
Decreased average CD4 +/CD8 + ratio[f]	1.2 (0.5–2.7)	0.2 (0.658)

[a] Confidence interval.
[b] Abnormally high alkaline phosphatase levels: > 150 IU/l (the high end of the normal range established by the North Carolina State University College of Veterinary Medicine).
[c] Abnormally high alanine aminotransferase levels: 45 IU/l (the high end of the normal range established by the North Carolina State University College of Veterinary Medicine).
[d] Abnormally high percentage of micronucleated binucleates: control group mean + 1 S.D.
[e] Abnormally high percentage of micronucleated binucleates: control group mean + 2 S.D.
[f] Abnormally low average CD4 + /CD8 + ratio = < control group mean − 1 S.D.

nal antibodies 8.534 or 12.125) and CD8 + (obtained by using monoclonal antibody 4.786) were variable in both the control and exposed groups. The average CD4 +/CD8 + ratios in the exposed animals were consistently lower than the ratios calculated for the control group; however, the differences were not statistically significant.

We examined the results from our biomarker assays to determine if any of the endpoints could identify an increased risk associated with the exposure (i.e. living in the community with the Superfund sites). The estimated relative risks of the exposed dogs (compared with the control dogs) and the Chi-square values for the biomarkers are presented in Table 4. Compared with controls, the relative risks (in exposed dogs) for abnormally high levels of serum alkaline phosphatase, alanine aminotransferase, or abnormally low CD4 + /CD8 + ratios were not statistically significant. In contrast, the relative risk for abnormally high PBL micronucleus frequency was statistically significant, suggesting that this was an appropriate biomarker for exposure to the contaminants in this area.

The application of biomarkers of exposure or effects in environmental epidemiology depends on the sensitivity and specificity of the particular endpoint(s) used to detect the exposures and outcome measures of concern. The sensitivity of a screening test refers to the probability of testing positive if the disease (or the change in the value of a biomarker) is really present. Specificity refers to the probability of testing negative if the disease is not present (Hennekens and Buring, 1987). We calculated the sensitivity and specificity (see Hennekens and Buring, 1987) for the chosen suite of biomarkers (using the control group values as our standard) to determine if they might serve as screening tests for potential exposure to organochlorine pesticides in the environment (see Table 5). In this study, serum levels of alkaline phosphatase and alanine aminotransferase and the CD4 + /CD8 + ratios were not sensitive enough to serve as screening tools for exposure; however, the PBL micronucleus test appeared to be a very sensitive and specific screening test for this exposure.

On the basis of our results from testing pet dogs in this community, the Agency for Toxic Substances and Disease Registry funded a study of the human effects from exposure to environmental contaminants in this community (see US DHHS, 1998). The study examined most of the biomarkers that we examined in the pet dogs (with the exception of serum chemistry) as well as immunoglobulin levels, serum pesticide levels, a skin test for delayed hypersensitivity to common antigens, and lymphocyte mitogen stimulation assays.

Table 5
Sensitivity and specificity of biomarkers used to assess exposure to environmental contaminants

Biomarker	Sensitivity	Specificity
Elevated alkaline phosphatase level[a]	0.15	0.15
Elevated alanine aminotransferase level[b]	0.15	0.15
Elevated percent micronucleated binucleates[c]	0.75	0.90
Elevated percent micronucleated binucleates[d]	0.70	0.95
Decreased average CD4 +/CD8 + ratio[e]	0.40	0.67

[a]Abnormally high alkaline phosphatase levels: > 150 IU/l (the high end of the normal range established by the North Carolina State University College of Veterinary Medicine).

[b]Abnormally high alanine aminotransferase levels: 45 IU/l (the high end of the normal range established by the North Carolina State University College of Veterinary Medicine).

[c]Abnormally high percentage of micronucleated binucleates: control group mean + 1 S.D.

[d]Abnormally high percentage of micronucleated binucleates: control group mean + 2 S.D.

[e]Abnormally low average CD4 + /CD8 + ratio = < control group mean − 1 S.D.

In general, the study found that most individuals had biomarker levels well within normal ranges (US DHHS, 1998) although there were some exceptions. For example, among people aged 40–59 years, there was a statistically significant increase in the percentage of micronucleated binucleates in those living within 1 mile of a specific contaminated site (1.9%) compared with those living further away from that site (1.4%, $P = 0.04$). Similar to the results in our study of pet dogs, residents who lived near one specific site had a lower mean CD4+/CD8+ ratio than residents who lived further away (1.9 vs. 2.2, respectively, $P = 0.05$). Also, red blood cell counts were slightly higher in males living in the Superfund community (mean $\pm$ S.D.) ($5.1 \pm 0.3 \times 10^6/\mu l$) than in males in the control community ($4.9 \pm 0.37 \times 10^6/\mu l$, $P = 0.05$). In the male dogs in our study, the red blood cell counts were similar in the two groups (mean $\pm$ S.D.) (controls: $6.8 \pm 0.67 \times 10^6/\mu l$, $n = 11$; exposed: $7.2 \pm 0.46 \times 10^6/\mu l$, $n = 4$).

4. Conclusion

As with other cross-sectional epidemiologic studies, there are limits to the conclusions that can be drawn from our study. Because each dog was examined only once, trends in biomarkers could not be determined. The assessment of exposure was qualitative and was based on historical evidence of widespread contamination in the study area (see EPA, 1990). Also, it is possible that higher levels of exposure in the past may have resulted in changes in biomarkers that were no longer detectable at the time of our study. Despite these shortcomings, we showed that pet dogs can be an early warning sentinel for human exposure to environmental contaminants. In addition, we demonstrated how the detection of changes in carefully chosen biomarkers in a population of sentinel animals could be an important first step in justifying a more detailed examination of potential exposure in the corresponding human community. Further investigations are needed to assess the sensitivity and reliability of using biomarkers in sentinel animal populations to identify and predict risks for adverse human health effects from exposure to environmental contaminants.

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