Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers

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Objective—To determine whether exposure to lawn or garden chemicals was associated with an increased risk of transitional cell carcinoma (TCC) of the urinary bladder in Scottish Terriers.

Design—Case-control study.

Animals—83 Scottish Terriers with TCC (cases) and 83 Scottish Terriers with other health-related conditions (controls).

Procedure—Owners of study dogs completed a written questionnaire pertaining to exposure to lawn or garden chemicals during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs.

Results—The risk of TCC was significantly increased among dogs exposed to lawns or gardens treated with both herbicides and insecticides (odds ratio [OR], 7.19) or with herbicides alone (OR, 3.62), but not among dogs exposed to lawns or gardens treated with insecticides alone (OR, 1.62), compared with dogs exposed to untreated lawns. Exposure to lawns or gardens treated with phenoxy herbicides (OR, 4.42) was associated with an increased risk of TCC, compared with exposure to untreated lawns or gardens, but exposure to lawns or gardens treated with nonphenoxy herbicides (OR, 3.49) was not significantly associated with risk of TCC.

Conclusions and Clinical Relevance—Results suggest that exposure to lawns or gardens treated with herbicides was associated with an increased risk of TCC in Scottish Terriers. Until additional studies are performed to prove or disprove a cause-and-effect relationship, owners of Scottish Terriers should minimize their dogs' access to lawns or gardens treated with phenoxy herbicides. (*J Am Vet Med Assoc* 2004; 24:1290–1297)

Transitional cell carcinoma (TCC) of the urinary bladder is the most common cancer of the urinary tract in dogs, with 1.2% to 2% of all cancers in dogs being TCCs of the urinary bladder.[1] While the incidence of TCC in the pet dog population is unknown, the prevalence of TCC in dogs examined at veterinary teaching hospitals in North America increased by > 600% between 1975 and 1995.[2] In that study,[2] the risk that Scottish Terriers would develop TCC was approximately 18 times the risk of mixed-breed dogs. Other breeds with a significantly increased risk of TCC, compared with mixed-breed dogs, were the Shetland Sheepdog (4.5 times), Wirehaired Fox Terrier (3.2 times), and West Highland White Terrier (3.0 times). This pattern of increased risk in terriers suggested a genetic predisposition.

The pathogenesis of TCC in dogs is probably multifactorial, involving both genetic and environmental determinants. A previous case-control study[3] of pet dogs of a wide variety of breeds found that TCC risk was unrelated to side-stream cigarette smoke and household chemical exposures, but was significantly increased in a dose-response manner in dogs exposed to topical insecticides, particularly flea and tick dips. This increased risk of TCC was further enhanced in overweight or obese dogs and in dogs living in close proximity to another potential source of insecticides, namely a marsh that had been sprayed for control of mosquitoes.

In 1991, an association was reported between an increased risk of malignant lymphoma in pet dogs and the owner's use of **2,4-dichlorophenoxyacetic acid (2,4-D)** herbicides in and about the home.[4] In this study, dogs belonging to owners that applied 2,4-D to their lawn or employed commercial lawn care companies to treat their yards ≥ 4 times/y had twice the risk of developing lymphoma, compared with dogs exposed to nontreated lawns. These findings were later challenged by a Chemical Industry Task Force,[5] and reanalysis of the original data, funded by an industry task force, failed to find a significant association between 2,4-D use and malignant lymphoma or a dose-response relationship.[6] A subsequent study,[7] however, demonstrated that dogs living in and around a residence recently treated with 2,4-D absorbed measurable amounts of the herbicide for several days after application. For example, dogs exposed to treated lawns during the preceding 7 days were 50 times as likely to have urine 2,4-D concentrations > 50 µg/L as were dogs that had been exposed to treated lawns > 7 days previously.

Phenoxy herbicides in general, and 2,4-D specifically, are among the most widely used chemicals in contemporary agriculture, and 2,4-D has been commercially available throughout the world for approximately 55 years.[8] The most convincing evidence suggesting that phenoxy herbicides are human carcinogens arises from epidemiologic studies of patients with non-Hodgkin's lymphoma.[9,10] However, lifetime cancer bioassays of rats, mice, and dogs have generally concluded that there was a lack of evidence of carcinogenicity, at least under experimental conditions.[11] The recent finding that

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Scottish Terriers had a significantly increased risk of TCC, compared with dogs of other breeds, afforded us an opportunity to use a spontaneously occurring cancer in an apparently genetically susceptible breed to test the hypothesis that natural exposure to phenoxy herbicides in general, and 2,4-D in particular, increases the risk of TCC. Specifically, the purpose of the study reported here was to determine whether exposure to lawn or garden chemicals was associated with an increased risk of TCC of the urinary bladder in Scottish Terriers.

Materials and Methods

Starting in June 2001, owners of Scottish Terriers with TCC (cases) and Scottish Terriers with other health problems, including other cancers (controls), were recruited through the Web site of the Scottish Terrier Club of America and through the Purdue Comparative Oncology Program. One of the authors (MHD) also contacted, by telephone or e-mail, individual Scottish Terrier owners and veterinarians known to be interested in Scottish Terriers. All potential participants were told the study was designed to collect information on potential risk factors for cancer, such as diet, water, medical history, medications, chemical exposures, and side-stream cigarette smoke. The specific study hypothesis was not revealed to the participants or recruiters, and questions were asked about a variety of environmental exposures.

Case dogs were Scottish Terriers in which TCC was diagnosed anytime after January 1, 1995. Dogs were included in the study as case dogs only if the owner submitted written proof of the diagnosis of TCC (ie, a histology report, cytology report, or both). Only 1 dog from a household could participate, even if TCC was diagnosed in > 1 dog in the household.

Control dogs were Scottish Terriers that were > 6 years old as of July 1, 1995, in which TCC had never been diagnosed (control dogs had to be > 6 years old because we expected that few case dogs would be < 6 years old). In addition, dogs were included as control dogs only if they did not have any history of urinary tract disease during the 2 years prior to entry into the study or death. Only 1 dog per household could serve as a control dog, and control dogs could not come from the same households as case dogs.

All case and control dogs enrolled in the study by January 31, 2003, were included in the analyses. The Purdue University Committee on Human Subjects approved all procedures used in this study by expedited review.

Owners of case and control dogs agreed to complete a written questionnaire pertaining to the dog's medical history and exposure to household lawn or garden chemicals during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs (ie, during the year before death or the year before entry into the study). A list of commonly used household, lawn, and garden chemicals, including brand names and active ingredients, was provided to the owners with the mailed questionnaire. Owners were instructed to consult the list to identify specific products they had used. Space was also provided for owners to write in the names of any commercial products they had used that were not included on the list. Owners were asked how often (never, sporadic, seasonal, or year-round) and the average number of times a year they used each product, as well as who treated the lawn or garden. In addition to their completed questionnaire, they were instructed to mail back the labels for lawn products they applied or to contact the commercial applicator for specific product information.

Types of lawn and garden pesticides used, determined on the basis of owner responses and product labels, were characterized as herbicides, insecticides, fungicides, algicides, acaricides, and molluscicides. A dog could possibly have been exposed to > 1 pesticide type or to > 1 brand-name product within each pesticide type. The active ingredient for each product was determined from owner-provided information or on the basis of trade name of the product, using the pesticide database of the Office of Indiana State Chemist.[12] Because a single brand could potentially contain > 1 active ingredient, exposure to > 1 herbicide was recorded, and each chemical was treated independently. Herbicide exposure was further grouped by chemical class as phenoxy acid, amino acid type, benzoic acid, dinitroaniline, picolinic acid, benzonitrile, or chloracetamide.[13] Each dog could potentially have been exposed to > 1 chemical class of herbicides. If owners were uncertain as to what specific product was used or if they provided incomplete label information, the exposure was considered as being of unknown type.

Data were analyzed with standard epidemiologic software.[14,a] Descriptive data were compared between case and control groups by use of χ^2 tests (categorical variables), independent-samples *t* tests (normally distributed continuous variables), or Mann-Whitney tests (non-normally distributed continuous variables). Each potential risk factor was examined for an association with TCC by means of univariate logistic regression[b] and the maximum likelihood method.[15,16] The association between potential risk factors and TCC was expressed as an **odds ratio (OR)** with 95% **confidence intervals (CIs)**. A test for a linear trend in the ORs was performed when appropriate.[14,c] Multivariate logistic regression was used to model the risk of TCC for potential risk factors with a P value < 0.05 in univariate analyses. The fit of multivariate models was determined by use of the Pearson χ^2 and Hosmer and Lemeshow statistics.[17]

Results

Eighty-three case and 83 control dogs were enrolled in the study. Fifty-two (63%) of the case dogs were dead at the time of enrollment in the study versus 10 (12%) control dogs. In 62 (74%) case dogs, the diagnosis of TCC was confirmed by means of histologic evaluation of tissue samples. In the remaining 21 (26%) dogs, the diagnosis was presumptive in that histologic examination of tissue samples had not been performed. In these dogs, the diagnosis was made on the basis of results of microscopic examination of a needle aspirate (12 dogs), cytologic examination of a urine sample (11 dogs), or cytologic examination of a bladder wash sample (1 dog). For 7 of these 21 dogs, diagnostic imaging (6 dogs) and tumor antigen testing (1 dog) were performed in addition to cytologic evaluation.

The most common health-related conditions affecting control dogs at the time of the study were cancer (20 dogs; 24%), skin disease (18; 22%), parasitic infections (16; 19%), idiopathic increases in hepatic enzyme activities (12; 14%), and scottie cramp (6; 7%).

Four (5%) control dogs had a history of urinary tract disease that had been treated successfully at least 2 years prior to enrollment in the study. In contrast, 27 (33%) case dogs were reported to have had signs compatible with chronic urinary tract disease within 2 years prior to the diagnosis of TCC.

Mean ± SD ages at the time of enrollment in the study for case and control dogs were 9.9 ± 2.0 years and 9.1 ± 2.3 years, respectively; case dogs were significantly (P = 0.01) older than control dogs. Thirty-four (41%) case dogs were male, as were 34 (41%) control dogs. All but 4 (5%) case and 15 (18%) control dogs had been neutered; a significantly (P = 0.04) higher proportion of control than of case dogs was sexual-

ly intact. One hundred forty-eight dogs (89%) were registered with the American Kennel Club.

Twenty (24%) case dog owners and 42 (51%) control dog owners indicated that their dogs had not been exposed to commonly used lawn or garden chemicals at their homes ($P < 0.001$; **Table 1**). Forty-two (51%) case and 15 (18%) control dogs had been exposed to herbicides, and 33 case (40%) and 20 (24%) control dogs had been exposed to insecticides. The 3 most common chemical classes of herbicides that dogs in the study were exposed to were phenoxy acids (34 [41%] case and 11 [13%] control dogs), represented by 2,4-D, 2-(4-chloro-2-methyl) phenoxy propionic acid (MCPP), and 4-chloro-2-methyl phenoxy acetic acid (MCPA); benzoic acids (20 [24%] case and 5 [6%] control dogs), represented by dicamba; and amino acids (18 [22%] case and 9 [11%] control dogs), represented by glyphosate. Many case and control dogs were exposed to herbicides belonging to more than 1 chemical class (**Table 2**). The owner of 1 control dog exposed to herbicides was not aware of the specific trade name or the common name of the herbicide used on his lawn by a commercial lawn care company.

Host factors found in univariate logistic regression analyses to be significantly associated with risk of TCC included age, neutering status, coat color, body weight, weight-to-height ratio, and having a first-degree relative with a history of TCC (**Table 3**). Univariate analysis also revealed that risk of TCC was higher for dogs with seasonal or year-round exposure to any lawn or garden (whether treated or not), compared with dogs with no or only sporadic exposure; for dogs with seasonal or year-round exposure to a lawn or garden treated with a herbicide; and for dogs with seasonal or year-round exposure to a lawn or garden treated with an insecticide (**Table 4**). In addition, the risk of TCC for dogs exposed to a lawn or garden treated with both a herbicide and an insecticide was higher than the risk for dogs exposed to a lawn or garden treated only with a herbicide or only with an insecticide.

Risk of TCC was significantly higher for dogs owned by individuals who used herbicides, regardless of whether the herbicides were applied by the owner or a commercial company (**Table 5**). The risk of TCC was also higher when the herbicide was applied seasonally or year round versus sporadically or never and when the herbicide was applied more often. In addition, risk of TCC was higher among dogs exposed to phenoxy acid herbicides (compared with dogs not exposed) or benzoic acid herbicides, but not among dogs exposed to amino acid type herbicides or to other types of herbicides. In general, risk of TCC was higher among dogs exposed to phenoxy acid herbicides or nonphenoxy acid herbicides, compared with dogs exposed to lawns or gardens to which no herbicides had been applied.

Two separate multivariate models were constructed. One model used information for all dogs regardless of whether the owners had reported access to a lawn or garden, while the second model used information only for those dogs reported to have had access to a lawn or garden. Since the pattern of risk was similar for the 2 models, only results for dogs with access to lawns or gardens were reported. Also, a separate multivariate model was developed including information only for confirmed cases of TCC. Since there was no appreciable difference between the models that included or excluded presumptive cases, only the model including all cases was reported. For all multivariate models, information regarding history of TCC in a first-degree relative was not included because this information was missing for 59 (71%) case dogs and 39 (47%) control dogs. Also, body weight was included in multivariate models rather than weight-to-height ratio because information on weight was missing for fewer dogs than was information on height.

Table 1—Exposure to common lawn and garden chemicals among 83 Scottish Terriers with transitional cell carcinoma (TCC) of the urinary bladder (case dogs) and 83 Scottish Terriers with other health problems (control dogs)

Pesticide	No. of case dogs (%)	No. of control dogs (%)
None	20 (24)	42 (51)
Herbicide	42 (51)	15 (18)
Insecticide	33 (40)	20 (24)
Fungicide	4 (5)	1 (1)
Algicide	1 (1)	1 (1)
Molluscicide	1 (1)	0 (0)
Acaricide	1 (1)	0 (0)
Unknown or uncertain	7 (8)	12 (14)

Owners were asked to indicate exposure to household lawn or garden chemicals during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. Values in each column do not add to 83 because some dogs were exposed to > 1 type of pesticide.

Table 2—Exposure to various herbicide active ingredients among 83 Scottish Terriers with TCC (case dogs) and 83 Scottish Terriers with other health problems (control dogs)

Active ingredient	No. of case dogs (%)	No. of control dogs (%)
None (not exposed to any herbicides)	34 (41)	56 (67)
2,4-D* only	2 (2)	1 (1)
MCPA* only	3 (4)	0 (0)
Glyphosate only	5 (6)	1 (1)
Pendimethalin only	0 (0)	1 (1)
Halosulfuran methyl only	1 (1)	0 (0)
Alachlor only	0 (0)	1 (1)
2,4-D and MCPP*	3 (4)	0 (0)
2,4-D and MCPA	1 (1)	0 (0)
2,4-D and glyphosate	1 (1)	0 (0)
2,4-D and dicamba	0 (0)	1 (1)
2,4-D and triclopyr	1 (1)	0 (0)
MCPA and triclopyr	1 (1)	0 (0)
2,4-D, MCPP, and glyphosate	1 (1)	2 (2)
2,4-D, MCPP, and dicamba	7 (8)	1 (1)
2,4-D, MCPA, and glyphosate	2 (2)	2 (2)
MCPA, dicamba, and pendimethalin	1 (1)	0 (0)
≥ 4 active ingredients	13 (16)	4 (5)
Unknown or uncertain	7 (8)	13† (16)

Owners were provided a list of commonly used lawn and garden chemicals and asked to indicate what chemicals their dogs had been exposed to during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs.

*Phenoxy acid herbicide class.

2,4-D = 2,4-Dichlorophenoxyacetic acid. MCPA = 4-Chloro-2-methyl phenoxy acetic acid. MCPP = 2-(4-Chloro-2-methyl) phenoxy propionic acid.

†Active ingredient and trade name were unknown for type of herbicide used on 1 control dog.

Table 3—Results of univariate logistic regression analysis of host factors potentially associated with risk of TCC in Scottish Terriers

Factor	No. of case dogs (%)	No. of control dogs (%)	OR (95% CI)	*P* value	*P* value for trend
Age (y)					0.003
≥ 4 to < 8	13 (16)	35 (42)	NA	NA	
≥ 8 to < 11	41 (49)	27 (33)	4.09 (1.84–9.11)	0.001	
≥ 11	29 (35)	21 (25)	3.72 (1.59–8.69)	0.002	
Sex					NA
Male	34 (41)	34 (41)	NA	NA	
Female	49 (59)	49 (59)	1.00 (0.54–1.86)	1.00	
Neutering status					NA
Sexually intact	4 (5)	15 (18)	NA	NA	
Neutered	79 (95)	68 (82)	4.36 (1.38–13.75)	0.01	
Coat color					NA
All or partly black	59 (71)	47 (57)	NA	NA	
Not black*	23 (28)	36 (43)	0.51 (0.27–0.97)	0.04	
Body condition					NA
Optimum	56 (67)	58 (70)	NA	NA	
Overweight	27 (33)	23 (28)	1.22 (0.62–2.37)	0.57	
Height (tertiles†)					0.30
First	9 (11)	17 (21)	NA	NA	
Second	21 (25)	31 (37)	1.28 (0.48–3.41)	0.62	
Third	18 (22)	20 (24)	1.70 (0.61–4.76)	0.31	
Weight (tertiles†)					0.04
First	23 (28)	35 (42)	NA	NA	
Second	22 (27)	24 (29)	1.40 (0.64–3.05)	0.40	
Third	33 (40)	23 (28)	2.18 (1.03–4.62)	0.04	
Weight-to-height ratio (tertiles†)					0.02
First	11 (13)	28 (34)	NA	NA	
Second	15 (18)	23 (28)	1.66 (0.64–4.31)	0.30	
Third	21 (25)	17 (21)	3.14 (1.22–8.10)	0.02	
Mating					NA
Outcross or nonselective	10 (12)	24 (29)	NA	NA	
Inbred or line bred	24 (29)	41 (49)	1.41 (0.58–3.43)	0.46	
First-degree relative with TCC					NA
No	8 (10)	30 (36)	NA	NA	
Yes	16 (20)	14 (17)	4.29 (1.49–12.37)	0.007	

Values in some categories may not add to 83 because of missing data.
*Includes brindle and wheaten coat colors. †Tertiles were sex specific.
OR = Odds ratio. CI = Confidence interval. NA = Not applicable.

Table 4—Results of univariate logistic regression analysis of potential associations between exposure to lawn or garden chemicals and risk of TCC in Scottish Terriers

Factor	No. of case dogs (%)	No. of control dogs (%)	OR (95% CI)	*P* value
Residence				
Urban	13 (16)	16 (19)	NA	NA
Suburban	51 (61)	49 (59)	1.28 (0.56–2.94)	0.56
Rural or farm	19 (23)	18 (22)	1.30 (0.49–3.45)	0.60
Access to lawn or garden (treated or nontreated)				
None or sporadic	9 (11)	19 (23)	NA	NA
Seasonal or year-round	74 (89)	64 (77)	2.44 (1.03–5.77)	0.04
Access to herbicide-treated lawn or garden				
None or sporadic	37 (45)	57 (69)	NA	NA
Seasonal or year-round	39 (47)	14 (17)	4.29 (2.05–8.97)	< 0.001
Access to insecticide-treated lawn or garden				
None or sporadic	47 (57)	56 (68)	NA	NA
Seasonal or year-round	29 (35)	15 (18)	2.30 (1.11–4.80)	0.03
Treatment of lawn or garden to which dog had access				
None	20 (24)	42 (51)	NA	NA
Insecticide only	13 (16)	14 (17)	1.95 (0.77–4.91)	0.16
Herbicide only	22 (27)	9 (11)	5.13 (2.00–13.15)	< 0.001
Both insecticide and herbicide	20 (24)	6 (7)	7.00 (2.43–20.13)	< 0.001
Exposure to insecticides indoors				
No	39 (47)	49 (59)	NA	NA
Yes	42 (51)	33 (40)	1.60 (0.86–2.97)	0.14

Owners were provided a list of commonly used lawn and garden chemicals and asked to indicate what chemicals their dogs had been exposed to during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. *See* Table 3 for key.

Table 5—Results of univariate logistic regression analysis of potential associations between exposure to herbicides and risk of TCC in Scottish Terriers

Factor	No. of case dogs (%)	No. of control dogs (%)	OR (95% CI)	*P* value
Individual applying herbicide				
None (no herbicides used)	34 (41)	56 (68)	NA	NA
Owner	25 (30)	9 (11)	4.58 (1.91–10.95)	< 0.001
Commercial company	14 (17)	4 (5)	5.77 (1.75–18.95)	0.004
Pattern of herbicide application				
None or sporadic	40 (48)	62 (75)	NA	NA
Seasonal or year-round	31 (37)	7 (8)	6.86 (2.76–17.08)	< 0.001
No. of applications/y*				
0	34 (41)	56 (68)	NA	NA
1–4	28 (34)	8 (10)	5.77 (2.36–14.09)	< 0.001
≥ 5	6 (7)	1 (1)	9.88 (1.14–85.56)	0.04
Phenoxy acid				
No	42 (51)	59 (71)	NA	NA
Yes	34 (41)	11 (13)	4.34 (1.98–9.54)	< 0.001
Benzoic acid				
No	56 (68)	65 (78)	NA	NA
Yes	20 (24)	5 (6)	4.64 (1.64–13.18)	0.004
Amino acid type				
No	58 (70)	61 (74)	NA	NA
Yes	18 (22)	9 (11)	2.10 (0.88–5.06)	0.10
Other herbicide†				
No	68 (82)	66 (80)	NA	NA
Yes	8 (10)	4 (5)	1.94 (0.56–6.76)	0.30
Class of herbicide				
None used	34 (41)	56 (68)	NA	NA
Not phenoxy acid‡	8 (10)	3 (4)	4.39 (1.09–17.70)	0.04
Phenoxy acid‡	34 (41)	11 (13)	5.09 (2.28–11.36)	< 0.001

*P value for trend, < 0.001. †Includes dinitroaniline, picolinic acid, benzonitrile, and chloracetamide. ‡Could include dogs exposed to another class of herbicides. Owners were provided a list of commonly used lawn and garden chemicals and asked to indicate what chemicals their dogs had been exposed to during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. *See* Table 3 for key.

Table 6—Results of multivariate logistic regression analysis of potential associations between exposure to lawn or garden chemicals and risk of TCC in Scottish Terriers

Factor	No. of case dogs (%)	No. of control dogs (%)	OR (95% CI)	*P* value
Age (y)*				
≥ 4 to < 8	11 (13)	32 (39)	NA	NA
≥ 8 to < 11	33 (40)	18 (22)	5.43 (1.99–14.86)	0.001
≥ 11	23 (28)	14 (17)	4.01 (1.38–11.63)	0.01
Neutering status				
Sexually intact	4 (5)	10 (12)	NA	NA
Neutered	63 (76)	54 (65)	2.07 (0.49–8.79)	0.32
Coat color				
All or partly black	50 (60)	39 (47)	NA	NA
Not black†	17 (21)	25 (30)	0.72 (0.29–1.79)	0.48
Weight (tertiles‡)§				
First	18 (22)	27 (33)	NA	NA
Second	19 (23)	16 (19)	1.40 (0.47–4.12)	0.55
Third	30 (36)	21 (25)	1.85 (0.72–4.79)	0.20
Access to lawns or gardens				
None or sporadic	5 (6)	8 (10)	NA	NA
Seasonal or year-round	62 (75)	56 (68)	1.69 (0.42–6.76)	0.46
Treatment of lawn or garden to which dog had access				
None	16 (19)	36 (43)	NA	NA
Insecticide only	13 (16)	13 (16)	1.62 (0.56–4.74)	0.38
Herbicide only	20 (24)	9 (11)	3.62 (1.17–11.19)	0.03
Both insecticide and herbicide	18 (22)	6 (7)	7.19 (2.15–24.07)	0.001

Owners were provided a list of commonly used lawn and garden chemicals and asked to indicate what chemicals their dogs had been exposed to during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. *See* Table 3 for key. Values in some categories may not add to 80 cases and 77 controls because of missing data.

*P value for trend, 0.01. †Includes brindle and wheaten coat colors. ‡Tertiles were sex specific. §P value for trend, 0.23.

When potential risk factors associated with risk of TCC in univariate analyses (ie, $P < 0.05$) were included in a multivariate model, the only host factor that remained significantly associated with risk of TCC was age (**Table 6**), with risk of TCC increasing as age increased. The risk of TCC was higher for dogs exposed to lawns or

Table 7—Results of multivariate logistic regression analysis of potential associations between exposure to herbicides and risk of TCC in Scottish Terriers

Factor	No. of case dogs (%)	No. of control dogs (%)	OR (95% CI)	*P* value
Age (y)*				
≥ 4 to < 8	11 (13)	32 (39)	NA	NA
≥ 8 to < 11	34 (41)	18 (22)	5.72 (2.10–15.54)	< 0.001
≥ 11	23 (28)	13 (16)	4.29 (1.48–12.48)	0.008
Neutering status				
Sexually intact	4 (5)	10 (12)	NA	NA
Neutered	64 (77)	53 (64)	2.11 (0.51–8.84)	0.31
Coat color				
All or partly black	51 (61)	38 (46)	NA	NA
Not black†	17 (21)	25 (30)	0.70 (0.29–1.69)	0.42
Weight (tertiles‡)§				
First	18 (22)	26 (31)	NA	NA
Second	20 (24)	16 (19)	1.42 (0.48–4.19)	0.52
Third	30 (36)	21 (25)	1.73 (0.67–4.50)	0.26
Access to lawns or gardens treated with insecticides				
None or sporadic	41 (49)	48 (58)	NA	NA
Seasonal or year-round	27 (33)	15 (18)	1.64 (0.69–3.86)	0.26
Class of herbicide				
None used	30 (36)	49 (59)	NA	NA
Not phenoxy acid¶	7 (8)	3 (4)	3.49 (0.67–18.12	0.14
Phenoxy acid¶	31 (37)	11 (13)	4.42 (1.74–11.19)	0.002

Owners were provided a list of commonly used lawn and garden chemicals and asked to indicate what chemicals their dogs had been exposed to during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. *See* Table 3 for key. Values in some categories may not add to 80 cases and 77 controls because of missing data.

**P* value for trend, 0.007. †Includes brindle and wheaten coat colors. ‡Tertiles were sex specific. §*P* value for trend, 0.30. ¶Could include dogs exposed to another class of herbicides.

gardens treated with both herbicides and insecticides than for dogs exposed to lawns or gardens treated with herbicides or insecticides alone. The fit of this model was found to be acceptable ($P = 0.70$; $R^2 = 0.25$). In a second multivariate model, exposure to lawns or gardens treated with phenoxy herbicides remained significantly associated with risk of TCC (**Table 7**), whereas exposure to lawns or gardens treated with nonphenoxy herbicides did not. For the host factors, only age remained significantly associated with risk of TCC. The fit of this model was also found to be acceptable ($P = 0.30$; $R^2 = 0.25$). Risk of TCC associated with exposure to individual pesticide types or individual chemical classes of herbicides could not be included in a single multivariate model because of excessive colinearity between these exposures. That is, many dogs exposed to 1 type of herbicide were also exposed to another type of herbicide.

Discussion

Results of the present case-control study provide evidence that the risk of TCC in Scottish Terriers exposed to phenoxy herbicides is 4.4 times the risk in Scottish Terriers without such exposure. Contact with nonphenoxy herbicides was also associated with an increased risk of TCC, but this increase was not significant. These findings suggest a gene-environment interaction for susceptibility to TCC and are consistent with findings of a recent meta-analysis[18] in which humans with a GSTM1 null genotype had 1.4 times the risk of bladder cancer. In humans, the product of the GSTM1 gene, glutathione S-transferase M1, is involved in detoxification of aromatic polycyclic hydrocarbons found in tobacco smoke, chemicals known to be associated with bladder cancer.[19] Scottish Terriers might have a similar gene that is responsible for an enzyme that detoxifies pesticide ingredients.

The median lethal dose (LD_{50}) of 2,4-D in dogs is approximately 100 mg/kg (45 mg/lb) and is lower than the LD_{50} in rats, mice, guinea pigs, and rabbits.[20] In dogs, phenoxy herbicides are absorbed mainly from the stomach and intestines following ingestion or grooming, with dermal absorption being slower and less complete.[21] Once absorbed, phenoxy herbicides are protein bound and rapidly distributed to the liver, kidney, and brain.[21] They are excreted through the renal tubules by an organic anion transport system, with most of the dose excreted unchanged in the urine.[21] The half-life of 2,4-D is approximately 18 hours.[21] Thus, dogs with daily or weekly exposure to lawns treated with 2,4-D might be expected to chronically excrete 2,4-D in their urine where it would come in constant contact with bladder epithelium. Phenoxy herbicides have been shown to depress ribonuclease synthesis, uncouple oxidative phosphorylation, and increase the number of hepatic peroxisomes.[21] However, whether any of these effects might initiate or promote development of TCC of the urinary bladder in Scottish Terriers is unclear, and further study is needed. In particular, research on the health effects of herbicides in dogs should shift from studies of acute toxicity to studies of chronic effects associated with long-term exposure, especially in genetically predisposed individuals.

Two phenoxy herbicides other than 2,4-D to which dogs in this study were exposed included MCPP and MCPA. The reported no observable-effect concentrations for these chemicals are 4 mg/kg (1.8 mg/lb) and 0.2 mg/kg (0.09 mg/lb), respectively, compared with a no observable-effect concentration for 2,4-D of 1 mg/kg (0.45 mg/lb) in dogs.[22,23] Carcinogenic activity

of these compounds in dogs has not been reported, and there is minimal published evidence implicating them as human carcinogens.[24]

There is convincing evidence that phenoxy herbicides are human carcinogens[9,10]; however, lifetime cancer bioassays of rats, mice, and dogs have not found any evidence of carcinogenicity.[11] In 1989, the Harvard School of Public Health convened an expert panel of scientists to examine the weight of evidence on the potential carcinogenicity of 2,4-D.[25] The predominant opinion among the panel members was that exposure to 2,4-D could possibly cause cancer in humans, although not all of the panelists believed the possibility was equally high.

A previous case-control study of TCC in dogs[3] found that the risk of TCC was associated with being overweight or obese and with the use of flea and tick dips. The authors suggested that the latter association could be attributable to inert ingredients, such as petroleum distillates, aromatic petroleum solvents, polyethers, and xylene, that often comprise 96% or more of flea and tick dips. Such substances are likely to be stored in body fat because of their lipophilic nature, and this may help explain the association between obesity and TCC. In the present study in which only Scottish Terriers were included, the risk of TCC also increased as body weight increased. This raises the possibility that inert ingredients, including solvents, emulsifiers, and spreaders, in lawn and garden pesticide products might be responsible. Many of these inert ingredients have adverse health effects and may themselves be used as pesticides. At least 382 chemicals on the US Environmental Protection Agency list of pesticide inert ingredients are or were once registered as pesticide active ingredients.[26] Eight inert ingredients are considered to be of toxicologic concern, and many others are potentially toxic.[26] While the identity of specific inert ingredients in a particular pesticide product is not available to the public, it has been estimated by the US Environmental Protection Agency that 1.2 billion pounds of conventional pesticides and 725 million pounds of wood preservatives are used each year in the United States.[27] If active ingredients represent 32% of the average pesticide product, about 4 billion pounds of inert ingredients are used each year. In comparison, in 1997, about 29 to 33 million pounds of 2,4-D were applied in the United States, and 2,4-D was ranked eighth on a list of commonly used active ingredients in pesticides.[28]

As with any retrospective study, there was a potential for recall bias among owners of case and control dogs included in the present study. However, because owners enrolled in this study were not aware of the specific hypotheses being tested when they completed the questionnaire, any recall bias would likely have been nondifferential with respect to case or control status. Also, as in a previous study of 2,4-D exposure and lymphoma risk in dogs,[4] owners may not have had accurate information regarding lawn products they used in the past. Recall error was minimized in the present study by providing owners with a reference list of commonly used lawn and garden products and by requesting that they either submit the actual package label from products they applied or contact the commercial applicator for this information. It is likely that any inaccurate recall of products used between owners of case and control dogs that did occur would have biased the OR toward 1 (ie, no association). Evidence against inaccurate recall in the present study is the finding that risk of TCC associated with exposure to herbicides used on lawns or gardens was high and was higher among dogs with seasonal or year-round exposure, compared with dogs with no or sporadic exposure. Also, the risk of TCC was higher for dogs exposed to phenoxy herbicides than for those not exposed to herbicides and for those exposed to nonphenoxy herbicides.

On the basis of the findings in this study, we recommend that owners of Scottish Terriers decrease their dogs' access to lawns or gardens that have been treated with pesticides, particularly phenoxy herbicides and possibly nonphenoxy herbicides as well, until additional risk studies have been conducted. In addition, we suggest that veterinarians discuss with owners routine (ie, every 6 months) cytologic examination of urine in Scottish Terriers > 6 years old and in other terriers. Genetic studies are needed to determine whether Scottish Terriers might have a gene that specifically predisposes them to TCC. Epidemiologic studies with genetically susceptible breeds of pet dogs could provide a humane alternative to experimental studies during the process of evaluating chemicals for human cancer risk.

[a]SAS, version 8.2, SAS Institute Inc, Cary, NC.
[b]Proc Logistic, SAS Institute Inc, Cary, NC.
[c]Proc GLM, SAS Institute Inc, Cary, NC.

References

1. Withrow SJ. Tumors of the urinary system. In: Withrow SJ, MacEwan EG, eds. *Small animal clinical oncology*. 2nd ed. Philadelphia: WB Saunders Co, 1996;380–392.

2. Knapp DW, Glickman NW, DeNicola DB, et al. Naturally-occurring canine transitional cell carcinoma of the urinary bladder: a relevant model of human invasive bladder cancer. *Urol Oncol* 2000; 5:47–59.

3. Glickman LT, Schofer FS, McKee LJ, et al. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. *J Toxicol Environ Health* 1989;28:407–414.

4. Hayes HM, Tarone RE, Cantor KP, et al. Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J Natl Cancer Inst* 1991;83:1226–1231.

5. Carlo GL, Cole P, Miller AB, et al. Review of a study reporting an association between 2,4-dichlorophenoxyacetic acid and canine malignant lymphoma: report of an expert panel. *Regul Toxicol Pharmacol* 1992;16:245–252.

6. Kaneene JB, Miller R. Re-analysis of 2,4-D use and the occurrence of canine malignant lymphoma. *Vet Hum Toxicol* 1999;41: 164–170.

7. Reynolds PM, Reif JS, Ramsdell HS, et al. Canine exposure to herbicide-treated lawns and urinary excretion of 2,4-dichlorophenoxyacetic acid. *Cancer Epidemiol Biomarkers Prev* 1994;3:233–237.

8. Ritter L. Report of a panel on the relationship between public exposure to pesticides and cancer. *Cancer* 1997;80:2019–2033.

9. Persson B, Dahlander AM, Fredriksson M, et al. Malignant lymphomas and occupational exposure. *Br J Ind Med* 1989;46: 516–520.

10. Hoar SK, Blair A, Holmes FF, et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA* 1986;256: 1141–1147.

11. Industry Task Force on 2,4-D Research Data. *Combined toxicity and oncogenicity study in rats: 2,4-dichlorophenoxyacetic acid: final report*. Vol 1. Vienna, Va: Hazelton Laboratories America Inc, 1986.

12. Pesticide Database Searches. Office of Indiana State Chemist Web site. Available at: www.kellysolutions.com/in/. Accessed May 15, 2003.

13. Monaco TJ, Weller SC, Ashton FM. *Weed science: principles and practices*. 4th ed. New York: John Wiley & Sons, 2002;183–197.

14. Dean AG, Dean AJ, Coulombier D, et al. *Epi Info, version 6: a word-processing, database, statistics program for public health on IBM-compatible microcomputers*. Atlanta, Ga: Centers for Disease Control and Prevention, 1995.

15. Allison PD. *Logistic regression using the SAS system: theory and application*. Cary, NC: SAS Institute Inc, 1999;5–84.

16. *SAS/STAT user's guide: version 8*. Vol 2. Cary, NC: SAS Institute Inc, 1999;1903–2042.

17. Hosmer DW, Taber S, Lemeshow S. The importance of assessing the fit of logistic regression models: a case study. *Am J Public Health* 1991;81:1630–1635.

18. Engel LS, Taioli E, Pfeiffer R, et al. Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review. *Am J Epidemiol* 2002;156:95–109.

19. Matanoski GM, Elliott EA. Bladder cancer epidemiology. *Epidemiol Rev* 1981;3:203–229.

20. Stevens JT, Sumner DD. Herbicides. In: Hayes WJ Jr, Laws ER Jr, eds. *Handbook of pesticide toxicology*. Vol 3. New York: Academic Press Inc, 1991;1317–1408.

21. Osweiler GD. *Toxicology*. Philadelphia: The Williams & Wilkins Co, 1996;257–265.

22. Yeary RA. Lawn care products. In: Bonagura JD, ed. *Kirk's current veterinary therapy XIII*. Philadelphia: WB Saunders Co, 2000; 221–222.

23. *Guidelines for drinking water quality. Health criteria and other supporting information*. 2nd ed. Vol 2. Geneva: World Health Organization, 1996;763–787.

24. Bond GG, Rossbacher R. A review of potential human carcinogenicity of the chlorophenoxy herbicides MCPA, MCPP, and 2,4-DP. *Br J Ind Med* 1993;50:340–348.

25. Ibrahim MA, Bond GG, Burke TA, et al. Weight of the evidence on the human carcinogenicity of 2,4-D. *Environ Health Perspect* 1991;96:213–222.

26. Knight H. Hidden toxic "inerts": a tragicomedy of errors. *J Pesticide Reform* 1997;10:10–11.

27. *Pesticide industry sales and usage: 1994 and 1995 market estimates*. Circular 733-R-97002. Washington, DC: Environmental Protection Agency, 1997.

28. Acquavella J, Doe J, Tomenson J, et al. Epidemiologic studies of occupational pesticide exposure and cancer: regulatory risk assessments and biologic plausibility. *Ann Epidemiol* 2003;13:1–7.