

Formulations of Deet, Picaridin, and IR3535 Applied to Skin Repel Nymphs of the Lone Star Tick (Acari: Ixodidae) for 12 Hours

J. F. CARROLL,¹ J. P. BENANTE,² M. KRAMER,³ K. H. LOHMEYER,⁴ AND K. LAWRENCE²

J. Med. Entomol. 47(4): 699–704 (2010); DOI: 10.1603/ME09239

ABSTRACT The efficacies of a 20% 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate (picaridin) spray, 20% 3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester (IR3535) spray, 20% picaridin lotion, 10% IR3535 lotion, and 33% *N,N*-diethyl-3-methylbenzamide (deet) cream in repelling nymphal lone star ticks, *Amblyomma americanum* (L.), were determined at 2-h intervals over 12 h using human subjects. A repellent formulation was applied in a 5-cm-wide band encircling a volunteer's lower leg. For each challenge, 70 host-seeking nymphs were released on each volunteer's ankle, and tick locations were recorded 10 min after the ticks were released. Ticks that crawled entirely across the repellent band were considered not repelled. For all formulations and time points, significantly fewer (all $P < 0.0001$) *A. americanum* nymphs crossed the treatment bands on the volunteers' ankles than crossed the corresponding area on the untreated control legs. Formulations containing $\geq 20\%$ active ingredient were highly effective, with $< 10\%$ of the ticks crossing through the treatment bands for any challenge during the 12 h. At least 40% of ticks exposed to any formulation for any challenge fell or crawled off the volunteers. There was no difference in effectiveness between the 20% spray and 20% lotion formulations of picaridin. The 10% IR3535 lotion was significantly less effective than the formulations with higher concentrations of repellent. In the formulations tested, deet, picaridin, and IR3535 provided lasting protection against *A. americanum*.

KEY WORDS *Amblyomma americanum*, 12-h duration, human volunteers, repellents

Although tick-borne pathogens can cause acute and chronic illnesses that can severely affect humans (Sonenshine 1993, Parola and Raoult 2001), implementation of area-wide tick control measures (Piesman and Eisen 2008) has been limited. By default, personal protective measures, such as applying repellents, are the recourse for persons venturing into tick habitats (CDC 2002, Debboun et al. 2007). For use on clothing, permethrin-based products have been the standard (Schreck et al. 1982, 1986; Lane and Anderson 1984; Evans et al. 1990); for use on human skin, products containing *N,N*-diethyl-3-methylbenzamide (deet) have dominated for several decades.

The lone star tick, *Amblyomma americanum* (L.), is of growing medical importance in the United States

(Childs and Paddock 2003), particularly in the southeastern and south central states. Furthermore, this species has been extending its range northward in the mid-Atlantic area (Ginsberg et al. 1991). It will readily bite humans and is known to transmit *Ehrlichia chaffeensis*, causative agent of human monocytic ehrlichiosis (Childs and Paddock 2003). *A. americanum* appears to be more difficult to repel than the black-legged tick, *Ixodes scapularis* Say (Carroll et al. 2004, 2007; Zhang et al. 2009), but the latter has greater notoriety as the vector of the causative agent of Lyme disease (Spielman et al. 1985) in the eastern and central United States. Lone star ticks engage in hunter-type host-seeking behavior (Waladde and Rice 1982). They are fast moving, readily attracted to CO₂ (Wilson et al. 1972), and tend to be more noticeable to the public than *I. scapularis* (Armstrong et al. 2001).

Deet has been tested against *A. americanum* in the field, where Solberg et al. (1995) found that 0.5 mg/cm² skin repelled 85% of the nymphs and adults during a 30-min exposure immediately after treatment, but only 55% were repelled during a 30-min exposure 6 h after treatment. Using fingertip bioassays, Pretorius et al. (2003) compared deet and picaridin (KBR 3023) and found that both repelled $> 85\%$ of *Amblyomma hebraeum* Koch nymphs at 1 h after application, and 71 and 54%, respectively, by 4 h posttreatment. In vitro bioassays, laboratory-reared and field-collected *A. americanum* nymphs have been shown to respond

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¹ Corresponding author: United States Department of Agriculture–Agricultural Research Service, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Agricultural Research Center, Beltsville, MD 20705 (e-mail: john.carroll@ars.usda.gov).

² Division of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD 20910.

³ United States Department of Agriculture–Agricultural Research Service, Biometrical Consulting Service, Beltsville, Agricultural Research Center, Beltsville, MD 20705.

⁴ United States Department of Agriculture–Agricultural Research Service, Knipping-Bushland United States Livestock Insects Research Laboratory, Kerrville, TX 78028.

similarly to the repellents deet and racemic 220 (Carroll et al. 2008a). Carroll et al. (2008b) tested cream formulations of 10 and 20% 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate (Bayrepel = picaridin) and SS220 (10 and 20%) and 33% deet (EDTIAR), the standard military repellent, against laboratory-reared *A. americanum* nymphs in a simulated field test in which at 2-h intervals volunteers stood for 5 min in large plastic tubs containing fallen leaves and 100 ticks. All formulations repelled >85% of *A. americanum* for 12 h, with 20% Bayrepel and 20% SS220 providing 100% protection for the entire period. Repellents may also be applied as sprays that require different formulations. In spite of its effectiveness in repelling mosquitoes and ticks, deet has been the subject of complaints about its feel on skin, absorption by skin, and as a plasticizer (Frances and Deboun 2007). The purpose of our study was to assess the efficacy of two spray formulations (20% picaridin, 20% IR3535) and two repellent lotions (20% picaridin, 10% IR3535) over 12 h postapplication against the EDTIAR (33% deet cream).

Materials and Methods

Ticks. Lone star tick nymphs used in this study were from a colony at the United States Department of Agriculture, Agricultural Research Service, Knippling-Bushland United States Livestock Insects Research Laboratory (Kerrville, TX), where they had fed on steers as larvae. At Knippling-Bushland United States Livestock Insects Research Laboratory, the ticks were held at 92–97% RH as larvae and 85% as nymphs, and all stages at 26.7°C and 14:10 h (L:D). One to 3 wk before testing, ticks were transferred from storage vials to 12-ml disposable syringes that were truncated at the nearest gradation to the tip. To transfer ticks to the syringe, the storage vial was placed in a porcelain tray with a band of masking tape (2.5 cm wide) coated with chalk on its inner side and a slit cut in the cloth screen capping the vial let controlled numbers of ticks escape. The syringe, with the piston retracted to about the 8-ml gradation, was connected to a pump aspirator. When 70 crawling nymphs had been collected, the syringe was separated from the aspirator and occluded with a perforated 7X Caplug (Caplugs, Buffalo, NY). The syringes were placed in plastic boxes containing moistened paper towels, and the boxes sealed in plastic bags. The ticks were held at 23°C, 97–99% RH, 16:8 h (L:D) photoperiod for 2–3 wk until tested.

Volunteers. Volunteers in this study were recruited, screened, and enrolled under a human-use protocol reviewed and approved by the Walter Reed Army Institute of Research (WRAIR) Institutional Review Board (WRAIR Protocol 1382: Laboratory Evaluation of Topical Arthropod Repellents against Ticks at WRAIR). Volunteers were recruited using flyers posted within the WRAIR. Interested parties were briefed on the nature of study participation. Participating volunteers signed informed consent forms before any study-related procedures in accordance with research guidelines for studies involving humans (Hu-

man Research Protection Office, United States Army Medical Research and Material Command, Ft. Detrick, MD). A total of 18 volunteers (six women, 12 men) participated in the study. The ages of volunteers ranged from 18 to 57, with a mean age of 29.7 yr. There was one minor adverse event and no deviations from the approved human-use protocol.

Repellents. Deet was formulated as the standard military use formulation (EDTIAR) by 3M (St. Paul, MN). The 10% IR3535 lotion and 20% IR3535 spray were formulated by EMD Chemicals (Darmstadt, Germany), and 20% picaridin lotion and 20% picaridin spray by Lanxess (Pittsburgh, PA). Because there was no carrier common to all the repellent formulations, the control was bare skin.

Design and Procedure. All five repellents were evaluated on human volunteers at six time points: 2, 4, 6, 8, 10, and 12 h postapplication. The first nine volunteers were each randomly assigned as follows: five to a repellent versus control regime, and four to a repellent versus EDTIAR regime (following the protocol used to power the study; see below). The remaining volunteers were randomly assigned to a repellent versus EDTIAR regime. Volunteers served as subjects on a day their personal schedule allowed, with a maximum of four subjects per day.

Two horizontal parallel lines, 5 cm apart, were marked with a fine-tipped marker around both legs \approx 5 cm above the ankle bone of each volunteer to indicate the band where the repellent was to be applied. The circumference of each leg in that band was measured to determine the area (cm²) to which the repellent was to be applied. The United States Environmental Protection Agency's Product Performance Test Guidelines recommend using 1–1.5 g of product (cream or lotion) and 1.0 g (aerosol or spray) per 600 cm² of skin surface. Based on these guidelines and the area calculated to be between the lines on the volunteer's legs, we determined the amount of repellent to apply to each volunteer. Repellents were applied with a nitrile-gloved hand (lotion and cream) or pipette (spray) to ensure a measured and even application over the surface to be treated. Once the repellent was applied, volunteers were instructed to engage in normal activities, but avoid actions that might rub the repellent off their legs onto other surfaces or their other leg. Applications were staggered at \approx 20-min intervals. At each postapplication time point (2, 4, 6, 8, 10, and 12 h), volunteers were challenged with ticks.

Footwear was removed at the start of each challenge. During the challenges, seated volunteers placed each foot in one of two (\approx 55 × 39 × 14 cm) plastic tubs (Sterilite, Townsend, MA) with a barrier of masking tape (7.6 cm wide) around their rims. An elliptical mirror (21.3 cm at widest; 18.7 cm at narrowest) was affixed to the tape barrier of each to afford each observer/tick remover a 360° view of the leg to which they were assigned. At a signal from the timer and data recorder, 70 ticks were released simultaneously onto the sloping medial aspect of each ankle of a volunteer 4–8 cm below the lower line marked on a volunteer's ankle. The Caplug was removed from the syringe, and

it was positioned nearly horizontally, with the open end touching the ankle. The piston was depressed (more slowly as the gasket approached the orifice), expelling the ticks onto the ankle. The syringe was rotated slowly to rub ticks onto the ankle. Any ticks that crawled on the outside of the syringe were transferred to the ankle with a small (000) white-bristled paintbrush. Ticks that crossed the upper boundary line of the treatment band were considered not repelled and removed immediately on masking tape, which was saved for counting the ticks. Ten minutes after the ticks were released, the numbers of ticks between the boundary lines of treatment band, on the ankle and foot (below the lower boundary line), and in the tub were collected on masking tape and counted according to location where captured. One pair of tubs was assigned to each volunteer for a given day. After the final challenge each day, the tubs were wiped with a wad of tissue soaked with 70% isopropanol.

Statistical Methods. Because the actual number of volunteers was not certain until the trials took place (volunteers could withdraw at any time), power was estimated, assuming that only nine volunteers were available and that the proportions of repelled ticks were near 0.7 (a 10% difference is more difficult to detect when proportions are nearer 0.5 than to 0 or 1), which may occur if repellent efficacy declines after many hours. We used a Monte-Carlo simulation (that is, we simulated the sort of data we expected to collect) to determine whether we could detect a true 10, 15, 20%, etc., difference. We used the R software (R Development Core Team 2008) with the glmmML package (Broström 2008), and allowed for substantial volunteer differences in attractiveness. With 70 ticks per leg, we could reliably (>80% power) detect a 15% true difference in proportions. This underestimated the power in this study because we anticipated more than nine volunteers, and these volunteers would be repeatedly tested the day they participated (so the volunteer effect would be better estimated).

We used a repeated measures incomplete block design, where volunteers were considered random (block) effects, and each volunteer tested with two repellent formulations, one on each leg or with a repellent on one leg and no treatment (bare skin) on the other leg, repeated over the course of the day six times. The two measures of tick responses that we analyzed were the proportion of ticks not repelled (i.e., those that completely crossed the 5-cm-wide band) and the proportion of ticks that dropped off the subject.

Because there were a number of potential covariates that might explain some of the variation seen in the responses, we first performed a stepwise analysis using the methods given in Kramer (2004) for mixed models, with volunteer and date as random effects. This method also helps determine the appropriate repeated measures (time series) covariance structure. The potential covariates were sex (of the volunteer), age, temperature, and relative humidity. The proportion of ticks was arcsine transformed before the procedure to satisfy the homogeneity assumption of anal-

ysis of variance. For the proportion not repelled, no covariates were indicated, and a simple autoregressive 1 (AR(1)) times series covariance structure was suggested. For the proportion dropped off, age was marginally significant, and a heterogeneous autoregression 1 (ARH[1]) covariance structure suggested (this differs from AR(1) in that variances can differ among testing time points).

Estimates of the two models were made using SAS proc glimmix (SAS Institute 2008) (the data are treated as samples from an overdispersed binomial distribution), with compound and time (as a qualitative variable), and their interaction as main effects, and other model components, as described above. We also altered the model slightly to test for linear effects of time for each compound (rather than treating time as a qualitative variable). Compounds were tested for significant differences within proc glimmix, using the Tukey adjustment on *P* values, and to obtain standard errors for compound-test time point means. Although diagnostics for generalized linear mixed models have not been adequately researched, proc glimmix does provide some residual diagnostic plots. On the link scale, residuals should be approximately normally distributed, and residual plots produced by proc glimmix for this study gave the appearance that the models fit the data well. However, all models demonstrated mild overdispersion, an indication that additional effects exist that were not included in the models; these could be things affecting tick responses, such as tick physiological age, health, or climbing ability, factors that may contribute to tick heterogeneity, but are difficult for researchers to assess.

Results

The linear predictor for the proportion of ticks that crossed the treatment band is written as follows in the standard format used for generalized linear mixed models: $f(p_{ijk}) = \mu + \tau_i + t_k + \pi_{ik} + \kappa_j$, where p_{ijk} is the modeled proportion of ticks crossing the treatment band, the function f is the logit, i indexes treatment (τ , fixed effect), k indexes time period (t , fixed effect), and j indexes volunteers (κ , random effect). As noted above, the residual correlation structure was modeled as AR(1) (the estimate of ρ was 0.39 with a standard error of 0.15); the overdispersion parameter estimated to be 1.86 (1.00 would indicate no overdispersion).

All repellent formulations allowed significantly fewer ($P < 0.0001$) *A. americanum* nymphs to cross the treatment bands on the volunteers' ankles than the untreated control. There was no significant diminution in efficacy for any formulation over time (Fig. 1). This was true whether time was entered as a categorical variable (as given in the equation above) or as a regression effect (i.e., βt , where $t = 2, 4, \dots, 12$), nor were there any significant interactions with treatment (all $P > 0.05$). Formulations containing $\geq 20\%$ active ingredient were highly effective in repelling the ticks throughout the 12 h, with fewer than 6% crossing through the treatment bands (Table 1). The propor-

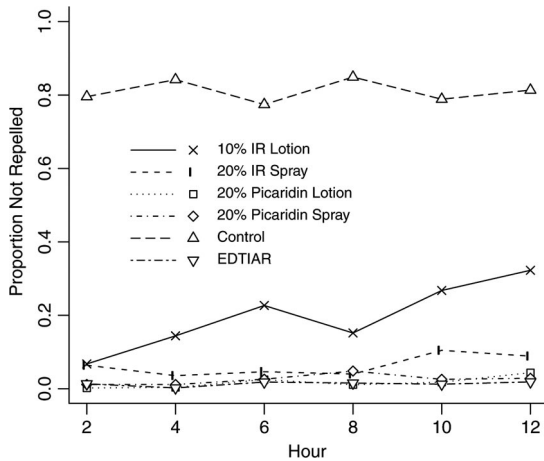


Fig. 1. Estimated proportion of *A. americanum* nymphs not repelled, i.e., ticks that crossed through 5-cm-wide band of repellent or untreated control on volunteers' lower legs within 10 min after ticks were released on volunteers' ankles.

tions of ticks not repelled by 20% picaridin lotion and 33% deet (EDTIAR) were only 0.0099 ± 0.0048 and 0.0114 ± 0.0027 , respectively. With 10% IR3535 lotion, <20% of the ticks crossed through the treatment band in all six challenges, but this was significantly more than for the formulations containing higher concentrations of repellent (Table 1). The proportions of ticks not repelled by 20% picaridin lotion and 33% deet (EDTIAR) were significantly lower than that of 20% IR3535 spray (Fig. 1). The 20% spray and 20% lotion formulations of picaridin were not significantly different ($P = 0.15$) in repelling *A. americanum*. Female volunteers had marginally higher ($P \approx 0.05$) numbers of ticks crossing through repellent-treated bands than male volunteers.

The equation for the linear predictor of ticks in tubs is similar to that for ticks crossing the treatment band, $f(p_{ijk}) = \mu + \tau_i + t_k + \tau t_{ik} + \kappa_j + \beta a_j$, where p_{ijk} is the modeled proportion of ticks crossing the treatment band, the function f is the logit, i indexes treatment (τ , fixed effect), k indexes time period (t , fixed effect), j

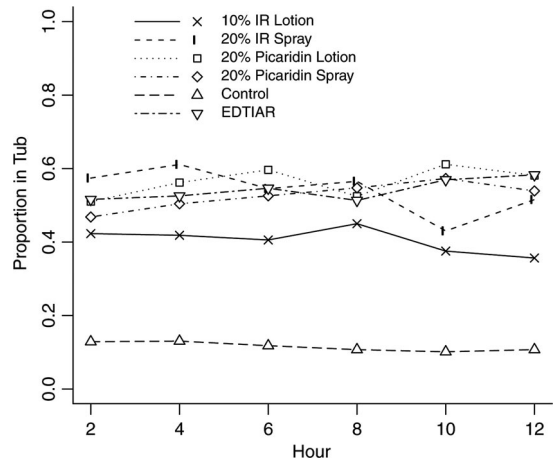


Fig. 2. Estimated proportion of *A. americanum* nymphs that had fallen or crawled off volunteers and were in the tub at 10 min after they were released on volunteers' ankles.

indexes volunteers (κ , random effect), and βa_j is the age effect (fixed) for subject j . During the model-fitting process, we refined the model as follows. The residual correlation structure was also AR(1) (estimate 0.38, standard error = 0.11), but the variances differed among time periods, although not in a systematic way (i.e., a different overdispersion parameter is fit for each time period).

Counts of ticks in the tubs (ticks that fell or crawled off the volunteer) at the end of each challenge showed that, for all repellent treatments, a mean of $\geq 40\%$ of the ticks left the volunteers (Fig. 2) with a significantly higher ($P = 0.016$) proportion of ticks leaving volunteers treated with 33% deet than volunteers treated with the 10% IR3535 spray (Table 1). No time effect was discerned in tub counts. However, there was an age effect, with higher tub counts with increasing volunteer age. The volunteer-to-volunteer variance (on the logistic scale) for ticks not repelled was 0.39 ± 0.15 and 0.22 ± 0.10 for ticks that were in the tub at 10 min after release.

Discussion

Using a bioassay similar to that in the current study, but that simulated host acquisition in the field, Carroll et al. (2008b) tested responses of *A. americanum* nymphs to repellent bands on volunteers' ankles for 12 h. However, only 33% deet cream (EDTIAR) was common to both the earlier and current study. In this study, 33% deet was highly effective, repelling a similar proportion of ticks to that repelled by 20% picaridin lotion, the highest among the treatments. Although this study lacked the more natural tick-host contact of the earlier trial in which ticks were released in tubs containing leaf litter, the placement of a large, known number of ticks directly on the volunteer facilitated a strong, uniform challenge to the repellent treatments with more easily interpreted results. Additionally, the relatively large number of volunteers ($n = 17$) allowed

Table 1. Estimated proportion of *A. americanum* nymphs not repelled, i.e. ticks that crossed through 5-cm-wide band of repellent or untreated control on volunteers' lower legs and proportion of ticks in the tub, i.e., ticks that fell or crawled off volunteer

Treatment	Formulation	Mean (\pm SE) estimated	
		Not repelled	In tub
Control		0.83 (0.04)a	0.11 (0.02)a
10% IR3535	Lotion	0.18 (0.05)b	0.40 (0.05)b
20% IR3535	Spray	0.06 (0.02)b, c	0.55 (0.05)b, c
20% picaridin	Spray	0.02 (0.01)c, d	0.53 (0.04)b, c
33% deet	Cream	0.01 (0.003)d	0.54 (0.02)c
20% picaridin	Lotion	0.01 (0.005)d	0.54 (0.05)b, c

Estimates are given averaging over six challenges because no significant loss in effectiveness was observed. Treatments listed in ascending order of effectiveness for "not repelled." Means in the same column followed by the same letter do not differ significantly ($P < 0.05$).

a thorough testing of the various repellent-formulation combinations.

The variation in tick responses to individual volunteers observed in the previous study (Carroll et al. 2008b) was again present, but less extreme. We detected a tendency for a greater proportion of ticks to cross through the treatment bands on female volunteers than on males, and more ticks crawled or fell off older volunteers. Variation in mosquito responses to individual volunteers has been well documented and associated with chemical cues (Costantini et al. 2001, Bernier et al. 2002). Golenda et al. (1999) found that among volunteers treated with the EDTIAR, female volunteers experienced less protection against mosquitoes over time than males, consistent with our results.

Fig. 1 shows an apparent slight increase in crossings of the band of 10% IR3535 lotion over time after application of the repellent; however, no increase was significant either as a linear or qualitative effect. The cream formulations (EDTIAR, 10 and 20% SS220, 10 and 20% picaridin) tested by Carroll et al. (2008b) in a simulated field trial also showed little decline in repellent activity against *A. americanum* over 12 h. Because the IR3535 spray and lotion treatments were of different concentrations, no conclusions can be drawn about which type application was more effective.

It is preferable for a repellent to cause ticks to leave a person completely rather than cause ticks to move from a treated portion of skin or clothing only to sequester themselves on nearby untreated or poorly treated skin or clothing. Counts of ticks in the tubs showed that the tested formulations had that capacity to varying degrees. Only 11% of the ticks released on the ankles designated as untreated controls were in the tubs at 10 min after their release. With the exception of 10% IR3535 spray, in which 18% of the ticks crossed through the treatment band, a little over half the ticks released left the volunteers treated with repellent. Interestingly, the proportions of ticks that left the volunteers treated with 20% IR3535 spray, 20% picaridin lotion, 20% picaridin spray, and 33% deet fell in the remarkably narrow range of 0.5267 and 0.5538. In fingertip and vertical filter paper bioassays, the more active *A. americanum* nymphs have also shown a greater propensity to drop from vertical surfaces than *I. scapularis* nymphs in response to repellent treatments (Carroll et al. 2010). Therefore, it should not be assumed that drop-off rates would be the same with other tick species.

In this study, the methods used for monitoring and removing ticks from volunteers at close range may have influenced in subtle ways the behavior (particularly ticks in the tub) of *A. americanum*, a species equipped with eyes (Phillis and Cromroy 1977) that responds quickly to host-produced cues. However, with 83% of the ticks progressing through the bands of the controls, the challenge to test formulations with this method appears robust. It is clear that formulations containing $\geq 20\%$ active ingredient provided long lasting protection under (mostly) controlled ambient

conditions, and that the spray and lotion formulations of picaridin were of equivalent efficacy. More strenuous physical activities may well decrease repellent longevity, because the treatments might be rubbed off or chemically altered by epidermal chemicals and perspiration. Comparisons of efficacies of tick repellents on volunteers engaged in greater physical activity than in the current study would provide a better understanding of repellency under so-called real world conditions.

Our results show that when appropriately formulated deet, picaridin and IR3535 offer lasting protection from tick bites. More needs to be learned about variation in individual attractiveness to ticks among humans.

Acknowledgments

We express our gratitude to the volunteers whose cooperation and patience were essential to the repellent trials. James McCrary and Abdul Saboor Khan (United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center), and Patrick Miles, Elizabeth Wanja, Chad Farmer, and Roxanne Flores (Walter Reed Army Institute of Research) contributed valuable assistance in preparing for and conducting this study. We also appreciate the efforts of Matthew Waldon (United States Department of Agriculture, Agricultural Research Service, Knippling-Bushland United States Livestock Insects Research Laboratory) in rearing the large number of ticks needed for the trials.

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Received 28 September 2009; accepted 26 February 2010.