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Effects of contamination of water with white phosphorus on drinking behaviour in sheep

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English summary

White phosphorus (WP) is a toxic contaminant found in many military training areas. Wild and domestic herbivores may be exposed to WP through drinking contaminated water. Knowledge of how animals react towards WP is important for developing risk assessment models. We investigated if contamination of water with WP affects drinking in domestic sheep. Six young rams were fed hay ad-libitum and given free choice between clean and WP contaminated water (1.5 mg WP/l) in a metabolism pen setup; quantities consumed were registered for three consecutive days (two 1.5 hour sessions per day). Data were analyzed using a linear model. The animals drank significantly more clean (473.1 ± 66.5 g) than contaminated (210.8 ± 46.3 g) water throughout a session. There was no effect of the interaction between treatment and day or between sheep and treatment, indicating that learning did not play any role and that differences in how individual sheep react to WP are negligible. The partial avoidance of WP contaminated water found in this study should reduce the risk of sheep being poisoned while grazing military training ranges; however, the danger of animals consuming particles suspended in water means that the risk is reduced less than the > 50 % reduction in drinking would otherwise imply.

Sammendrag

Hvitt fosfor er et giftig stoff som kan forekomme i skyte- og øvingsfelt. Husdyr og vilt kan bli eksponert for hvitt fosfor gjennom å drikke vann forurenset med hvitt fosfor. Kunnskap om hvordan dyr reagerer på hvitt fosfor i miljøet er viktig i forbindelse med å gjøre risikovurderinger av denne typen forurensning. Det ble undersøkt hvorvidt vann forurenset med hvitt fosfor kan påvirke inntak av vann hos sau. Seks unge værer ble foret med høy *ad-libitum* og ble gitt frihet til å velge mellom vann forurenset med hvitt fosfor (1.5 mg hvitt fosfor/l) og rent vann i en metabolismeboks hvor mengden vann konsumert ble registrert over tre påfølgende dager (to 1,5 timers bolker). Dataene ble analysert ved å benytte en lineær modell. Dyrene drakk statistisk signifikant mer rent vann (473,1 ± 66,5 g) enn forurenset vann (210,8 ± 46,3 g) gjennom forsøket. Det var ingen effekt av interaksjonen mellom behandling og dag og mellom sau og behandling, noe som indikerer at læring spilte ingen rolle og forskjeller i hvordan individuelle sauer reagerer på hvitt fosfor er neglisjerbar. Den delvise unngåelsen av vann forurenset med hvitt fosfor skal kunne redusere risikoen for sau som beiter i skyte- og øvingsfelt, men faren for at dyrene kan få i seg partikler av hvitt fosfor suspendert i vannet gjør at risikoen blir redusert mindre enn den > 50 % reduksjonen i drikking skulle tilsi.

Content

Preface

This study was performed under the project 108903, Munitions; pollution, environmental risk and remediation. The study was performed under a cooperation between FFI and the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås. The authors Geir Steinheim, Øystein Holand and Tormod Ådnøy work at the Nowegian University of Life Sciences.

1 Introduction

White phosphorus (WP) is a toxic compound used in smoke, tracer, illumination and incendiary munitions. In Norway WP is only used in smoke munitions. In areas used for military training WP may be deposited in the environment when burn is incomplete, and may contaminate soils and ponds (Voie et al., 2010). In Norway, most training ranges for heavy weaponry are situated in remote areas that are often grazed by large herbivores. Wild ungulates but also free-ranging livestock may then be exposed to WP. When deposited into an anaerobe environment, i.e. in water, wet snow or waterlogged soils or wetlands etc., WP is quite stable and may be present in the environment for a long time (Walsh et al., 1996). In an Alaskan wetland used for military training waterfowl deaths continued 15 years after use of WP ceased (Walsh et al., 2006). WP is soluble in water only to about 3 mg/l at 20°C (IPCS, 2010). In addition, it will often be present as suspended particles in contaminated water.

In vertebrates, waterfowls seem to have the highest incidence of WP poisoning (Sparling et al., 1997; Sparling et al., 1998), but WP-related deaths have been reported also in domestic sheep (*Ovis aries*) (Steward, 1930) and musk oxen (*Ovibos moschatus*) (Tørnes, 1988). WP poisoning of ungulates could thus be an animal welfare problem, but knowledge of incidence and of how ungulate behaviours affect the risk is currently non-existent. Obviously, there is little knowledge about wild ungulate mortality on an individual level; the same is the case for free-ranging sheep on Norwegian rangelands. However, carcasses are rarely found in shooting ranges were WP has been used.

Will sheep and other ungulates use water sources that are contaminated with WP, or will they avoid them, absolutely or to some degree? While having no evolutionary relationship with this manufactured compound animals may still react to novelty of smell and taste (e.g. Villalba et al., 2006) and they may develop responses through post-ingestive feedbacks (Provenza, 1995) . Sensory cues of WP may also resemble other compounds to which animals have evolved responses and phosphorus itself is a mineral that is involved in a wide range of metabolic processes (Ternouth, 1991). Knowledge about the avoidance/preference behaviour towards WP will be an important input in the risk assessment of grazing animals in contaminated areas (Voie et al., 2010).

In this preliminary study an experimental approach is used to determine if drinking in sheep is affected by WP contamination, when sheep are allowed to choose between clean water and water in which WP is solved. In Norway free-ranging sheep may be exposed to WP on the range; we also regard the sheep as a relevant model animal for other large wild and domestic ungulates.

2 Materials and Methods

2.1 Study animals and experimental setup

The study was approved by the Norwegian Animal Research Authority (NARA) before start-up. None of the animals suffered any ill effects from participating in the study. The experiment was conducted at the Department of Animal and Aquacultural Sciences in Ås, Norway.

Six 9 ½ month old rams, weighing between 50 and 60 kg, of the composite breed Norwegian White sheep (see Oklahoma State University (2010) for description of the main sub-breed within Norwegian White, the Dala) were used as study animals. The rams were placed in separate metabolism pens, each extended with a larger pen where the water sources were placed (see Figure 2.1 for details).

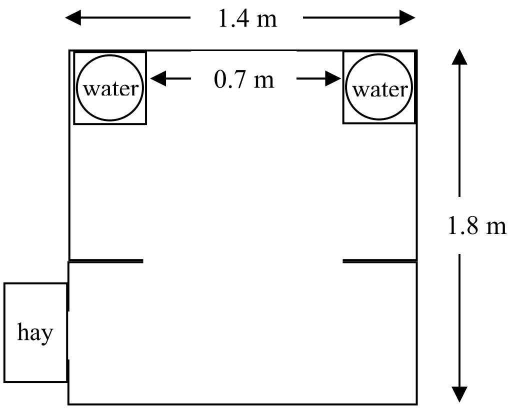

Figure 2.1 Extended metabolism pen. One water source contained clean water, the other water contaminated with white phosphorus (1.5 g/l). Positions of clean and contaminated water were swapped halfway through the day (Photo; Kristin B. Bruun, UMB).

The sheep were familiar with the general setup from a previous forage preference experiment (not involving WP), and were also allowed two days to get re-familiarized with the setup. Drinking water was offered in buckets placed within plywood boxes designed to keep feces and urine away from the water.

The experiment was conducted in June 2009, inside a large, well ventilated room, with approximately 1.5 m between pens. The weather was unusually warm, and daytime temperatures inside the room varied between 18 and 25°C during the experiment. On the three consecutive experimental days one bucket was filled with 2.0 l clean water, the other with 2.0 l of a filtered water-white phosphorous solution (1.5 mg WP/l). We randomized the starting positions of clean and contaminated water within day and pen, and halfway through the day the positions were switched. Water content in the buckets was registered at the start at 1000 hrs, when positions were switched (1130 hrs), and when the day's experiment was ended (1300 hrs). If at any time the amount of clean water was reduced to below ≈ 1.0 liter we added a measured quantity of clean water (drawn just before the day's experiment started and stored in open buckets within the experimental room) to approximately the same level as in the WP-solution bucket. Water had to be added in three instances; the level of contaminated water never had to be replenished.

Figure 2.2 Sheep in an extended metabolism pen choosing between clean and contaminated water (Photo: Kristin B. Bruun, UMB).

The animals had free access to good quality hay throughout their stay in the pens. When not choosing between clean and contaminated water the lambs had free access to clean drinking water. As would be the case on rangeland pastures the rams were offered salt (NaCl) but not multi-mineral supplements two weeks before (spent on pasture) and during the experiment.

2.2 Statistical analysis

A general fixed linear model was chosen for the analysis, using the GLM procedure in SAS version 9.1.3 (SAS Institute Inc., 2004). In addition to F-tests, means were calculated for the effects in the model. Data were perfectly balanced, so calculating corrected means - LSMEANS - give exactly the same values as the means.

The model that was used:
Intake = treatment + day + session + treatment × day + sheep + sheep × treatment + e,

where Intake is the amount of water (in g) consumed from one of the two water sources per animal and session, treatment is either "clean water" or "water with white phosphorus"), day is one of the three days (categorical), and session is first period (1000 – 1130) of a day or last period of a day (1130 – 1300), defined as categorical. The interaction between treatment and day, treatment*day, has six levels and was added to account for the possibility of day having a different effect on intake of clean versus contaminated water.

Study animal was entered as a fixed effect – sheep – and represents one of the six study animals (categorical); sheep × treatment (12 levels) was added to see if individual sheep reacted differently to the treatment. Finally, e is the residual error, implicitly defined as the variation within day, time of day, sheep and treatment.

3 Results

On average (± SE), a sheep drank 342.0 (± 43.1) g water from each bucket during a 1.5 hour session; mean intake of WP contaminated water and clean water was 210.8 (± 46.3) g and 473.1 (± 66.5) g, respectively. The model explained ≈ 40 % of the variation in water intake (R^2= 0.395). Coefficient of variation (CV) was 94.5. Treatment (clean or contaminated water) had a highly significant effect on intake (df= 1, F= 11.86, p= 0.001), with sheep drinking more from the clean water buckets. Day also affected water intake (df= 2, F= 5.71, p= 0.006), mainly because the animals drank more during first day.

The other effects in the model did not have significant effects; time of day (df= 1, F= 1.74, p= 0.192), the interaction between treatment and day (df= 2, F= 0.79, p= 0.461), sheep (df= 5, F= 0.98, p= 0.440), and sheep by treatment (df= 5, F= 0.87, p= 0.51). Means for all levels of the effects are presented in Table 3.1. In nine out of the 36 one and a half hour long sheep sessions (25 %) did a sheep drink more contaminated than clean water; in three of the 18 sheep days (17 %) was the total daily intake of WP higher than that of clean water. All individual sheep drank more clean water than contaminated water when all days were pooled.

Effect	N	Level	Mean	Std.dev.
Grand mean	72	.	342.0	365.5
Treatment	36	Clean	473.1	398.1
	36	WP	210.8	277.7
Day	24	1	522.4	441.9
	24	2	231.1	304.0
	24	3	272.4	270.0
Time of day	36	Session 1	392.3	346.2
	36	Session 2	291.7	382.1
Treatment*Day	12	Clean Day 1	708.8	460.7
	12	WP Day 1	336.1	346.9
	12	Clean Day 2	368.4	354.4
	12	WP Day 2	93.8	162.5
	12	Clean Day 3	342.3	274.4
	12	WP Day 3	202.6	257.8
Sheep	12	1	481.7	545.5
	12	2	333.7	260.4
	12	3	274.0	304.0
	12	4	404.0	412.4
	12	5	221.8	237.6
	12	6	336.7	362.8
Sheep*treatment	6	sheep 1 Clean	738.3	591.7
	6	sheep 1 WP	205.0	347.6
	6	sheep 2 Clean	394.5	175.6
	6	sheep 2 WP	272.8	330.9
	6	sheep 3 Clean	360.7	370.5
	6	sheep 3 WP	187.3	219.0
	6	sheep 4 Clean	490.7	356.5
	6	sheep 4 WP	317.3	478.6
	6	sheep 5 Clean	297.7	284.8
	6	sheep 5 WP	146.0	171.0
	6	sheep 6 Clean	537.0	376.9
	6	sheep 6 WP	136.3	226.4

Table 3.1 Mean water intake per session for the six sheep, with standard deviations (Std.dev.), for all effects in the model. For the effect of Treatment, "Clean" is clean water, "WP" is water contaminated with white phosphorous.

4 Discussion

The results indicate it being unlikely that sheep will actively seek out WP contaminated water sources on heterogeneous rangelands. Depending on how clean and contaminated water sources are distributed in the grazing area it is also unlikely that sheep will show a total WP avoidance out on the range. The study animals consumed approximately twice as much clean water as WP contaminated water, but the partial avoidance of WP did not increase or decrease within or between days, indicating that learning through post-ingestive feedback was not important. The sheep kept sampling and consuming contaminated water throughout the experiment. A similar pattern was found by Kronberg (2008) for sheep and cattle choosing between tap water and water contaminated with tannin.

In our study the low concentration of P_4 explain why learning (Villalba et al., 2006) seem not to have taken place. With a maximum potential daily intake of P through contaminated water of ≈ 0.015 g the P intake through forage, approximately 5-7 g per animal and day, should completely dominate the animals' choices where P is concerned. Also, our animals had only three days to learn, and the setup with clean and contaminated water simultaneously available did not facilitate rapid learning. The effect of WP on sheep behavior found in this study means that sheep will have a reduced risk of being poisoned while grazing military training ranges contaminated with WP compared to a case of no effect. It is doubtful that there will be a reduced risk when clean water is not readily available to the animals. The reduction in risk is assumed to be smaller than the halving of drinking of WP solutions found here. The main danger of WP is likely to be large particles suspended in the water. Especially if such particles are mixed in with a surface layer of froth/foam one mouthful may be fatal. Animals may also cause particles on the bottom of shallow water to be temporarily suspended and thus consumed.

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References

IPCS. International Chemical Safety Card: ICSC 0628, Phosphorus (Yellow). International Programme on Chemical Safety. IPCS; 2004. Available at: http://origin.cdc.gov/niosh/ipcsneng/neng0628.html [accessed 22.Jan. 2010].

Kronberg, SL. Intake of water containing condensed tannin by cattle and sheep. Rangeland Ecology and Management 2008;61:354-8.

Oklahoma State University. Breeds of Livestock – Sheep Breeds. Available at: http://www.ansi.okstate.edu/breeds/sheep/ [accessed 22.Jan. 2010].

Provenza, FD. Post-ingestive feedback as an elementary determinant of food preference and intake in ruminants. Journal of Range Management 1995;48:2-17.

Racine, CH, Walsh, ME, Roebuck, BD, Collins, CM, Calkins, D, Reitsma, L, et al. White phosphorus poisoning of waterfowl in an Alaskan salt-marsh. Journal of Wildlife Diseases 1992;28:669-73.

SAS Institute Inc. SAS OnlineDoc® 9.1.3. Cary, NC, SAS Institute Inc. Available at: http://support.sas.com/documentation/onlinedoc/91pdf/index_913.html [accessed 21.Jan 2010]

Sparling, DW, Gustafson, M, Klein, P, Karouna-Reiner, NK. Toxicity of white phosphorus to waterfowl: Acute exposure in mallards. Journal of Wildlife Diseases 1997;33:187-97.

Sparling, DW, Day, D, Klein, P. Acute toxicity and sublethal effects of white phosphorus in mute swans, *Cygnus olor*. Archives of Environmental Contamination and Toxicology 1998;36:316–22.

Stewart, WL. A curious case of phosphorus poisoning in sheep, Journal of the Ministry of Agriculture (Great Britain) 1930;37:56-9.

Tørnes, JA. Bestemmelse av hvitt fosfor i prøver fra Forsvarets skytefelt på Dovre, FFI/Rapport-6009; 1988.

Villalba, JJ, Provenza, FD, Hall, JO, Peterson, C. Phosphorus appetite in sheep: dissociating taste from postingestive effects. Journal of Animal Science 2006;84:2213-23.

Voie, ØA, Johnsen, A, Strømseng, A, Longva, KS. Environmental risk assessment of white phosphorus from the use of munitions: a probabilistic approach. Science of the Total Environment, *in press* – accepted 04-January-2010.

Walsh, ME, Collins, CM, Racine, CH. Persistence of white phosphorus (P-4) particles in salt marsh sediments. Environmental Toxicology and Chemistry 1996;15:846-55.

Walsh, ME, Zufelt, JE, Collins, CM. Remedation of white phosphorus contamination in an Alaskan wetland. Journal of Environmental Engineering and Science 2006;5:87-93.