Impact of Drinking Water Treatment on Poultry Health and Performances: An Experimental Study

¹Cherifa Boumedous, ^{2,3}Zouhir Djerrou and ³Youcef Hamdi Pacha

¹Department of Biological Sciences, Faculty of Sciences, University of Oum Bouaghi, Algeria ²Department of Sciences of Nature and Life, Faculty of Sciences, University of August 20th 1955, Skikda, Algeria ³Laboratory of Pharmacology and Toxicology, University of Mentouri Constantine 1, Algeria

Article history Received: 31-12-2016 Revised: 03-02-2017 Accepted: 04-02-2017

Corresponding Author: Youcef Hamdi Pacha Laboratory of Pharmacology and Toxicology, University of Mentouri Constantine 1, Algeria Email: hamdpacha@yahoo.fr **Abstract:** The present study aimed to investigate the impact of water quality on broilers performance. Two flocks of 10 chicks each were separated and maintained in the same poultry barn in Hamma Bouziane (Constantine). The animals were given standard food in the same husbandry conditions. The first group was provided untreated well water; however, the second one was given water treated by sodium hypochlorite. The water was analyzed for physicochemical and bacteriological characteristics at days 0 and 42 in the two flocks. The growth of chicks was investigated each week until 42th day. The results obtained has revealed that water untreated was contaminated and does not meet the standards recommended by WHO, it led to a decrease in performance represented by a very significant decrease in weight from the 7th to the 42nd day. The study demonstrates the importance of drinking water treatment in the success of broiler breeding.

Keywords: Physicochemical Water Parameters, Chlorination, Broilers, Antibacterial Treatments, Performance

Introduction

Water is the most important nutrient for poultry; birds generally drink approximately twice as much water as the amount of feed consumed on a weight basis (Blake and Hess, 2001). When temperature is high, water consumption is three times increased and water quality has emerged as one of the factors explaining water use, episodes of diarrhea but also the criterion on which a large margin of progress is possible (Travel *et al.*, 2006). Animals that cannot drink properly (insufficient quantity and quality of water) will tend to eat less, therefore grow less quickly and thus be less productive.

In poultry farms, digestive pathologies are more and more difficult to control by breeders. These digestive disorders, especially enteropathies, as well as an enteritis will cause moistening of the droppings, this degrades the state of the litter which becomes difficult to manage and which leads to an ammonia production which irritates the respiratory system; it is then easy to be wrong of target when treating a respiratory disease! Thus, litter degradation can be related to digestive disorders before 42 day (diarrhea or nonspecific enteritis) whose agents can be infectious agents. Indeed, there is a relationship between the technical performances and the management of the watering by the breeder, animals has to develop pathologies that will have an economic impact. In the case of a microbial or viral infection, the intestinal wall may be affected as a result of digestive disorders, which mainly result in enteritis (Hoerr, 1988). These pathologies generally result in increased secretion of water and electrolytes and inflammation in the intestinal mucosa causing excretion in the litter of undigested food fractions. These profuse diarrheas moisten litter (Ruff et al., 1981; Hoerr, 1988). A degraded litter favors the development of coccidia which can be the cause of a decrease in live weight in the adult and a decrease in growth in the young. Also, deteriorated litter has a direct impact on the locomotor system of animals (lameness, sternum ulcers) with an impact on carcass quality (increase in seizure rate, decrease in yield, lesions of the breast). So the first point to consider is the impact of the bacteriological and physico-chemical quality of water on poultry farming, the use of pipettes limits this risk, but does not solve upstream chemical and microbiological pollution. The presence of micro-organisms in water is caused by the contamination of wells and springs by fecal matter (presence of enterococcal fecal streptococci) or by fouling of distribution networks, which then become privileged places of multiplication bacterial growth (Guérin et al., 2011). The second point is to ensure a high level of protection for humans. Contaminated livestock can also contaminate other



© 2017 Cherifa Boumedous, Zouhir Djerrou and Youcef Hamdi Pacha. This open access article is distributed under a Creative Commons Attribution (CC-BY) 3.0 license.

farms, but also humans directly or the food they consume (Montiel, 2007).

The third point is the application of a mandatory disinfectant that is indispensable and obligatory to remove bio-residual bacterial films not eliminated by stripping and to prevent the reconstitution of a bio-film, especially Salmonella, from residual bacteria (Itavi, 2004).

The current study aims to investigate the impact of the bacteriological and physico-chemical quality of water on broiler chicken performances.

Materials and Methods

Experimental Protocol

This study was conducted on two lots of broiler chickens of 10 each, which were monitored weekly, from day 1 to 42 days. The two lots are placed in the same building, separated and under a common environment and with administration of the same foods for both lots in quantity and duration. The chicks were controlled at days: 1, 7, 14, 21, 28, 35 and 42, the weight was recorded each visit. The source of water supply was different for the two batches: A batch of 10 animals was supplied untreated well water; the second batch (10 animals) was supplied with treated and controlled water obtained from public water supply network system (circuit water). Complete bacteriological analyzes were carried out to ascertain the quality of the water at the inlet and the outlet of the pipes at the level of the breeding vessel and at the last drinking trough, with a partial bacteriological analysis at the end of the line. The samples of water were collected in dark sterile glass bottles of 250 and 1000 mL, which were transferred at 4°C to microbiological laboratory of Dakssi (Constantine) (Papadopouloua et al., 2008). The bacteriological quality of water was performed by the membrane filter technique as described in Standard Methods (APHA, 1998) to investigate the following parameters: Total flora, Total Coliforms (TC), coliforms TT, Faecal Streptococci (FS), sulfite-reducing bacteria and Salmonella according to procedures described elsewhere (Papadopouloua et al., 2008).

Full and partial physico-chemical analyzes were also carried out (every 15 days). Day 1 analyzes at the building and at the last watering trough allows us to know the state of cleanliness of the pipelines, because knowing the content of micro-organisms determines the state and thickness of the bio-film, which serves as support for pathogens. The other bacteria sought are pathogenic and are a sign of contamination of water. The measurements of free chlorine and pH are carried out at each visit to the last feeding trough; to verify if the treatment products added at the level of the building end up (Travel *et al.*, 2007), the water temperature was measured each visit by a thermometer at the drinking troughs.

In order to check the performance of the growing birds, an individual weighing was carried out for all the animals, every week, taking care to handle the subjects in calm (Traore, 2015).

Statistical Analysis

The results of animal weights were presented as mean with their variances. The data were analysis by *one* way Anova to compare the differences between the two groups; the level of significance was set at of $p \le 0.05$.

Results

Results of Physicochemical Analysis of Water

As shown in Table 1, well water (supplied for flock 1) has higher nitrate and iron content than recommended standards as well as the pH and the hardness; these parameters are relatively high that standards mentioned in Table 2 (WHO, 1998).

For the second group, chicks were supplied with water coming from the public water distribution network, hence, normally the assurance of water of proper quality (Table 3). Thus the physicochemical analysis of tap water indicates that it is hard water. A continuous processing is performed on the water by sodium hypochlorite (NaCl).

Bacteriological Analysis Results

In qualitative terms, the sanitary quality of the water is low because it does not meet the standards recommended by the WHO (1998). So, there is a biological pollution linked especially to fecal coliforms and fecal streptococcus, well water was not protected and no antibacterial treatment is introduced for improving its quality (Table 4).

For second flock (Table 5), we notice the presence of aerobic germs in tap water which corresponds to bacterial development suspended in pipes with organic matter, this total flora decreases from the 28th day, this decrease is visible only from the 42nd day because of progressively injected and controlled chlorine.

In some cases of water rechlorination at much higher chlorine levels to those used for water intended for human consumption: 0.2 to 0.3 mg L^{-1} of chlorine for humans and 2 to 3 mg L^{-1} of chlorine for poultry, because birds are bacteriologically more sensitive, a good water quality is needed.

Zootechnical Performances of Flocks

By comparing the weight of the two flocks of broilers with different watering source (Table 6), one flock is watered by treated drinking water and the other flock is watered by untreated drinking water. A significant difference was found in weight between the two flocks with a better gain in weight for the flock whose source of water was treated. The results of physicochemical and bacteriological analyzes and the efficiency of the treatment of water with the reduction of bacterial load (Total coliforms), disappearance of pathogenic bacteria, hence the disappearance of bio-film with the broilers weight increase, all these results suggest that treated water is a factor of a real broiler breeding success.

|--|

Complete physicochemical analysis				Partial analysis			
Date of sampling	Well water 1 st day	Circuit water 1st day	Water from the trough 1st day	Water from the trough On the 14th day	Water from the trough On the 28th day	Water from the trough On the 42th day	
PH	7,7	8	8	8,5	8,5	8,5	
Temperature	19°C	24°C	27°C	26°C	28°C	27°C	
Hardness	72,5	ND	ND	ND	ND	ND	
Nitrate (mg/l)	80	ND	ND	ND	ND	ND	
Iron(mg/l)	1	ND	ND	ND	ND	ND	

ND: Not detected

Table 2. Standards parameters of water for human consumption and for breeding (AFSSA, 2005)

Standards parameters	Potability for human	Satisfactory water for breeding
Temperature *	≤ 15°C	16°C
pH	6.5 < pH <8.5	6.5< pH <7.5
Hardness (HT)**	Soft: $8^{\circ}F \le HT < 15^{\circ}F$	
	Hard: $15^{\circ}F \le HT < 30^{\circ}F$	10 to 15°F
Nitrate	$<50 \text{ mg L}^{-1}$	50 mg L^{-1} + with a margin up to 80 mg L^{-1}
		(if the water is bacteriologically appropriate)
Iron	$<0.2 \text{ mg L}^{-1}$	$<0.2 \text{ mg L}^{-1}$

* For reasons of organoleptic quality, maintain drinking water at a temperature of 15°C or below (Pangborn and Bertolero, 1972). ** Hardness was given in degrees French (°F)

Table 3. Physicochemical analysis of water of the second flock
Complete physicochemical analygicDertial physic

	Complete physicochemical analysisPartial physicochemical analysis						
Date of sampling	Public water 1st day	Circuit water 1st day	Water from the trough 1st day	Water from the trough 14th day	Water from the trough 28th day	Water from the trough 42th day	
PH	7,1	7	6,5	6,7	6,7	6,9	
Temperature	25°C	26°C	24°C	25°C	25°C	21°C	
Hardness	42	ND	ND	ND	ND	ND	
Nitrate (mg/l)	50	ND	ND	ND	ND	ND	
Iron(mg/l)	0	ND	ND	ND	ND	ND	

ND: Not detected

Table 4. Bacteriological analysis of collected water (flock 1)*

	Complete physicochemical analysis			Partial bacteriological analysis			Bacteriological standards recommended
Date of sampling	Well water 1st day	Circuit water 1st day	Water from the trough 1st day	Water from the trough 14th day	Water from the trough 28th day	Water from the trough 42th day	in poultry farming (Itavi, 2001; Villate, 2001)
Total flora	300	600	600	800	800	800	<100 (in 100 mL)
Total coliforms	500	1100	1100	1100	1100	1100	<5 (in 100 mL)
Coliformes TT	0	13	13	16	16	16	<5 (in 100 mL)
Fecal streptococci Sulphite-reducing	6	6	<10	<10	<10	<10	<5 (in 100 mL)
bacteria	absence	absence	absence	absence	absence	absence	< 2 (in 20 mL)
Salmonella	absence	absence	absence	absence	absence	absence	0 in 51
Water treatment	No treatment	No treatment	No treatment	No treatment	No treatment	No treatment	

*Group of chicks supplied with untreated water

Table 5. Destarials sized analysis of callested system (fleals 2)*

	Complete	physicochem	nical analysis	Partial bacteriological analysis			Bacteriological standards recommended
Date of sampling	Public water 1st day	Circuit water 1st day	Water from the trough 1st day	Water from the trough 14th day	Water from the trough 28th day	Water from the trough 42th day	in poultry farming (Itavi, 2001; Villate, 2001)
Total flora	30	300	300	300	100	<3	<100 (in 100 mL)
Total coliforms	absence	absence	absence	absence	absence	absence	<5 (in 100 mL)
Coliformes TT	absence	absence	absence	absence	absence	absence	<5 (in 100 mL)
Faecal streptococcus	absence	absence	absence	absence	absence	absence	<5 (in 100 mL)
Sulphite-reducing bacteria	absence	absence	absence	absence	absence	absence	< 2 (in 20 mL)
Salmonella	absence	absence	absence	absence	absence	absence	0 in 51
Water treatment	0	0	0.3	0.2	0.2	0.2	

*Experimental group (water treated by sodium hypochlorite)

Table 6. Weights of b	proilers of the two	groups during	6 weeks
-----------------------	---------------------	---------------	---------

	Weight (g) (floo	k 1) untreated water	Weight (g) (flock	Flock 1 Vs flock 2		
Age (day)	Mean	Variance	Mean	Variance	P value	
1	58	4,444	57,5	6,944	0,64504	
7	109	8,88	155,55	27,777	$1,46549E^{-14}$	
14	211,55	39,527	405,111	28,861	0	
21	408,5	4,285	605,22	22,694	0	
28	709	7,142	811,111	111,111	4,996E ⁻¹⁴	
35	1008,5	4,285	1112,44	94,027	9,99201E ⁻¹⁵	
42	1299,75	16,5	1412,444	94,777	6,88338E ⁻¹⁵	

Discussion

Physico-Chemical Quality of well Water and Mains Water

The temperature of the water is between 19 and 28°C; these high temperatures can be explained by the influence of the ambient temperature on the water, but also the underground water table, itself being at a shallow depth. It should be noted that water with a temperature between 25 and 28°C is a good culture medium for environmental microorganisms (Makoutode et al., 1999). For Vaillant (1973), a temperature of 25°C favors the metabolism of mesophilic species. A high water temperature in the network of 18°C or water tarps can be the basis of a major planktonic development (Bouziani, 2000). It should be mentioned that the use of fresh water to combat heat stress can have a positive effect; studies have shown that giving fresh water to chickens contributes to their daily weight gain (Olkowski, 2009). It is also pointed out that the lowering of the temperature below the norms lead to a slowing down of the molecular agitation and a less dispersion of the chlorine molecules (Bouziani, 2000).

The results of analyzes show that well water and mains water have a high hardness, this high hardness is generally due to an excess of calcium, magnesium, manganese and iron (Itavi, 2001). Our water has a hardness of more than 15°F as recommended by the WHO (WHO, 1998), it significantly increases the total

flora concentration at start-up and the risk of contamination by germs and this is consistent with our results and results found the study of Travel *et al.* (2006).

The very calcareous waters can easily lead to a scaling of the pipes (Keck, 1995), accidents of breeding, precipitation of salts in the watering equipment, which can decrease the flow of water and potential water deprivation. Water deprivation has adverse effects on poultry growth rates with increased morbidity and mortality and may disrupt antibacterial treatment (Itavi, 2004; Olkowski, 2009).

The pH is an indicator on the acidity or basicity of water, the water of the present study has a basic pH; it is water calcareous or soiled by organic matter, this has been demonstrated by Keck (1995).

At day 0, a pH greater than 6 promotes the development of the bio-film in the pipes upstream of the building and in the building. At day 30, a PH higher than 7 significantly increases the risk of contamination of drinking water by potentially pathogenic bacteria, which has been demonstrated in our analyzes. However, pH is probably the most important factor to consider when assessing water quality. Our results are consistent with those obtained by Travel *et al.* (2006).

It should be emphasized that a water rich in organic matter and iron would promote the progression of the bacterial load, the presence of organic matter in the drinking troughs and in the pipes is common and associated with a high temperature will promote the development of fecal germs such as Streptococci and coliforms and Salmonella (Itavi, 2004), in fact the formation of bio-film in pipelines can relegate bacteria that will contaminate water (Pupin *et al.*, 2013), without forgetting the neutralization of the treatment applied (Itavi, 2006).

The chemical results of water collected during our study reveal the presence of chemical elements, pollution index such as nitrates and iron; nitrates are an indicator of organic or fecal pollution (Villate, 2001). High concentrations of nitrate are generally due to contamination, non-leakage of wells, permeable soils with deep water table, located in intensive farming areas or in infiltration sites of septic tanks. A recent study by Zaki *et al.* (2004) found that the presence of nitrate in drinking water can alter the functions of thyroid hormones, which can have a negative impact on growth rate. As a result, high nitrate levels may cause digestive problems and low growth.

Bacteriological Quality of Water

Bacteriological analysis has shown that well water is more contaminated than network water and shows bacteriological pollution related mainly to total coliforms, fecal coliforms and fecal streptococci. The presence of these contamination germs may be due to a failure of disinfection, contamination of the reservoir, work on the network (Joffin and Joffin, 1999). This pollution increases greatly in drinking troughs or the formation of a bio-film. The behavior of the breeders (the non respect of the rules of hygiene), the factors related to the mode of water management (collection, transport, storage). Analysis of total coliforms provides information on the health quality of water and the possible vulnerability of a source to surface pollution and are essential in the diagnosis of fecal contamination (Rodier et al., 2009). Therefore, the main source of water supply to the building is a risk factor for the initial contamination of water by the indicator flora (potentially pathogenic germs). The wells vehicles more germs than the public grid.

Treatment with Sodium Hypochlorite

The bacteriological analyzes carried out show the effectiveness of the treatment; at 35 and 42 days, the presence of the flora indicator is significantly reduced with antibacterial treatment. Permanent chlorination makes it possible to reduce the development of the total flora to the last watering place at 35 and 42 days. Compliance with these recommendations for the presence of a buffer tank, a product/water contact time of 20 min and control of the residual doses up to the last watering point, can destroy 100% of the germs present in the water. Compliance with these recommendations for efficient chlorination and regular control at the end of the line is associated with a reduced contamination of the pipelines by the bio-film at 35th day (Leclerc *et al.*,

1977; Leclerc and Mossel, 1989). The bacteriological treatment significantly reduces the risk of water contamination, early disinfection (<2 weeks of chicken age) or in case of diarrhea, reduces the risk of water contamination at the last watering place on day 35 according to Travel *et al.* (2007) and this was demonstrated in our study.

It should be noted that the presence of fecal streptococci in the livestock building and in the drinking troughs at start-up is mainly due to the storage conditions that are involved. Tank and circuit and reservoir water, in the case of untreated water and even more with chlorinated water in which the level of free chlorine has disappeared completely, the stagnation of water in the reservoirs causing dramatic increases in total bacteria ranging from 10 to 10,000 times that initial number, this disturbance may further affect the enumeration at 20°C. In water distributed in networks, the microorganisms tend to deposit and then to attach themselves to the pipes constituting a bio-film (Haslay and Leclerc, 1993). Our bacteriological results are closed to the results of the study obtained by Itavi (2006). The type of material constituting the pipes, before the building and between the building and the drinkers, influences the proportion of total flora present at start-up. The polyethylene of high density constituting the pipes going from the building to the drinkers decreases the amount of bio-film found at start up to the beak of the bird will always be less than 1000 CFU/100 mL.

The filter placed before the treatments, its change and its regular washing, reduce the risk of contamination by germs at the end of the line at 35th day thus reducing the presence of the bio-film in the pipes. The early appearance of diarrhea (as of the 10th day of age) for the first batch (untreated water) suggests that the presence of bio-film in the pipes at start-up is a factor favoring the appearance of digestive disorder from the first days. This was proved in our study.

Conclusion

The quality of drinking water is an important factor that may increase the transmission of pathogenic germs to animals. In some cases, water can cause substantial mortalities in intensive farming, to the point where rechlorination of tap water is even crucial. The microbiological quality of drinking water should be supervised by analysis and regular controls to ensure its safety.

Deterioration in performance at the beginning as well as heavy economic losses caused by the degree of digestive disorders, require the respect of good hygienic practices during the breeding period, this can keep the barn clean of germs, prevent the endangerment of the broilers' health status and reduce digestive imbalances.

Acknowledgement

We would like to thank all the persons who helped us to carry out this work in its different stages.

Funding Information

No necessary funding information.

Author's Contributions

Cherifa Boumedous: The first author designed the study, assisted sample collection, data analysis and manuscript preparation.

Zouhir Djerrou: Participated in manuscript preparation and results discussion.

Youcef Hamdi Pacha: Designed and supervised the study and participated in results interpretation.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that no ethical issues involved.

References

- AFSSA, 2005. French food safety agency. 3rd legislature of 01.01.2006.
- APHA, 1998. Microbiological Examination, Part 9000. In: Standard Methods for the Examination of Water and Wastewater, Clesceri, L.S., A.E. Greenberg and A.D. Eaton (Eds.), American Public Health Association, Washington, ISBN-10: 0875532357, pp: 1220.
- Blake, J.P. and J.B. Hess, 2001. Evaluating water quality for poultry. ANR.
- Bouziani, M., 2000. Notion de la potabilité de l'eau: l'eau de la pénurie aux maladies.
- Guérin, J.L., D. Balloy and D. Villate, 2011. Poultry diseases.
- Haslay, C. and H. Leclerc, 1993. Micribiology of drinking-water. In: Microbiologie Des Eaux D'alimentation, Haslay, C. and H. Leclerc (Edn.), Tec & Doc-Lavoisier, Paris, ISBN-10: 2852069180, pp: 495.
- Hoerr, F.J., 1988. Pathogenesis of enteric diseases. Poultry Sci., 77: 1150-1555. PMID: 9706081
- Itavi, 2001. The quality of water has direct impact on poultry health. Sci. Technical Rev.
- Itavi, 2004. Water in poultry farms. Poultry Sci. Technol., 1086: 10-11.
- Itavi, 2006. Poultry raising and digestive disease of turkey chick. Edition Science and Technology in Animal Products.
- Joffin, C. and N.J. Joffin, 1999. The Waters: In microbiology alimentary.
- Keck, G., 1995. Quality of breeding water and hygienic of the food chain. Vet. Rev., 146: 815-820.

- Leclerc, H. and D.A.H. Mossel, 1989. The main processing steps. Techniques documentations-Lavoisier Paris.
- Leclerc, H., R. Buttiaux, J. Guillaume and P. Watter, 1977. Disinfection of water.
- Makoutode, M., A.K. Assani, E.M. Ouendo, V.D. Agueh and P. Diallo, 1999. Qualité et mode de gestion de l'eau de puits en milieu rural au Benin: Cas de la sous-préfecture de Grand-Popo. Médecine d'Afrique Noire, 46: 528-534.
- Montiel, A., 2007. The quality of water in poultry.
- Olkowski, A.A., 2009. La qualité de l'eau d'abreuvement du bétail: Guide de terrain relatif aux bovins, aux chevaux, à la volaille et aux porcs.
- Pangborn, R.M. and L.I. Bertolero, 1972. Influence of temperature on taste intensity and degree of liking of drinking water. J. Am. Water Works Assoc., 64: 511-515.
- Papadopouloua, C., V. Economoua, H. Sakkasa, P. Gousiaa and X. Giannakopoulosc *et al.*, 2008. Microbiological quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates. Int. J. Hyg. Environ. Health, 211: 385-397. PMID: 17728184
- Pupin, P., J. Leorat and A. Dronneau, 2013. Qualité de l'eau. La lettre Synthèse élevage.
- Rodier, J., B. Legube, N. Merlet and R. Brunet, 2009. L'analyse de l'eau. Eaux naturelles, eaux résiduaires, eau de mer, 9e éd. Technique et ingénierie, Dunod, Paris.
- Ruff, M.D., P.C. Augustine and P.A. Madden, 1981. *Eimeria meleagrimitis*, *E. adenoeides* and *E. dispersa*: Severity of infection and changes in the intestinal mucosa of the turkey. Exp Parasitol., 51, 1: 87-94.
- Traore, A.O., 2015. Guide technique et économique d'un élevage de poulets de chair. Harmattan Mali.
- Travel, A., I. Bouvarel, D. Chevalier, L. Fulbert and S. Garnier, 2006. Conduite d'élevage et pathologies digestives des dindonneaux-Quels facteurs maîtriser pour limiter l'impact des entérites non spécifiques? Étude de cas. Rapport OFIVAL.
- Travel, A., D. Chevalier, F. Merlet and L. Fulbert, 2007. Facteurs de variation de la qualité bactériologique de l'eau en élevage de dindes. Septièmes Journées de la Recherche Avicole, Tours, 28: 537-540.
- Vaillant, J.R., 1973. Protection de la qualité des eaux et maîtrise de la pollution.
- Villate, D., 2001. Maladies Des Volailles. 3rd Edn., France Agricole, Paris, ISBN-10: 2855574668, pp: 576.
- WHO, 1998. European standard for drinking water. World Health Organization.
- Zaki, A., A. Ait Chaoui, A. Talibi, A.F. Derouiche and T. Aboussaouira *et al.*, 2004. Impact of nitrate intake in drinking water on the thyroid gland activity in mal rat. Toxicol. Lett., 147: 27-33.