Contaminants in eggs: effects of feed and environment

C.A. (Kees) Kan

Animal Sciences Group of Wageningen UR, Lelystad, The Netherlands

kan@dronten.net

Introduction

Chlorinated pesticides and (halogenated) environmental contaminants can enter laying hens in a variety of ways. The most important way is via the feed (including soil intake). However, direct application on the animal and contact with the skin as well as inhalation via the airways should not be ruled out.

Most components of animal feed are digested and absorbed during passage trough the intestinal tract. Digestion and absorption of nutritive components have been extensively studied. In contrast, digestion and absorption of unwanted substances like dioxins, pesticides, heavy metals, drugs and polycyclic hydrocarbons is often not considered. Yet, they are often analyzed both in feeds and eggs. Some scientists assume complete absorption of these noxious substances, as a worst-case scenario to predict residues in eggs from those in feed. By doing so, they ignore the physiological processes occurring during transit through the intestine, absorption into the general circulation and intermediary metabolism. Furthermore this approach does not take advantage of the existing knowledge, which can help to identify or implement possible control points for reduction of levels of residues in eggs.

Specific reviews on residues in eggs are rather scarce and often limited in their scope. The Egg report (DiGanghi and Petrlík, 2005) as well as Schoeters and Hoogenboom (2006) e.g. concentrate on dioxins and PCBs, while van Overmeire et al. (2006) concentrate on free-range eggs from Belgium. Kan(2005) specifically paid attention to residues in free range or organic systems. Kan & Mulder (1997) have given a general account of substances likely to be present in eggs. The recent review by Kan & Meijer (2007) gives a general insight on transfer of toxic substances from feed to food.

The control of targeted and suspected egg samples in the EU - most recently reported for 2005 - (http://ec.europa.eu/food/food/chemicalsafety/residues/workdoc_2005_en.pdf) shows that residues of some chlorinated pesticides, PCBs and a number of drugs and coccidiostats have been found and that the number of non-compliant results dropped in further in 2005 as compared to 2004 and 2003. Origin of the contamination source is often the feed. Drug and coccidiostat residues originate generally from improper production or storage procedures. Pesticide and PCB residues come with contaminated (fat containing) feedstuffs (Dórea, 2006).

This paper aims to review the possibilities for chemical contamination during egg production. It intends further to corroborate some of these possibilities with data from the literature.

Risk assessment and communication

The general assumption - for non-carcinogenic substances – is, that a threshold limit exists below which exposure of humans to chemical residues does not pose a threat to human health. This limit expressed as an acceptable or daily intake (ADI), however, is set often for individual compounds and does not take into account possible interactions between different chemicals neither aggravating nor alleviating. The concept however does still allow certain conclusions on the risks of human exposure to chemicals via the food. Nasreddine & Parrent-Massin (2002) e.g. reviewed the risks of intake of certain pesticides via plants and heavy metals via food in general. Pesticide intake was quite low compared to the tolerances but the intake of certain consumer groups in Europe of heavy metals proved to be near or above the assumed safe levels. They concluded that more attention should be paid to high risk food items and to a more solid support of the tolerances, as a number of assumptions are still part of the calculations. Pussemier et al. (2006) attempted to compare the chemical safety of foodstuffs produced in Belgium. They report a scarcity of objective data essential to carry out a meaningful risk comparison.

Risk management has not been addressed widely yet. Kan and Meijer (2007) have attempted to indicate possible risk management strategies for the different groups of toxic substances present in animal feed.

Next to risk evaluation and management is risk communication, leading to perception by both authorities and the general public.

Winter and Francis (1997) already indicated 10 years ago, that risk information could be characterized as one-way and technocratic and thus it formed a barrier in effective public involvement. Trautman (2001) gave a nice overview on the importance of risk communication in the perspective of risk analysis as a whole. Shaw (2003) noticed a decline of trust (by the public) in government, science and the food industry. Remarkably, younger people expressed more overall trust in science and the government to provide them with facts about food safety. On the other hand, the informal word of mouth seemed to be a key source of both negative and positive information about food issues. A recent study in Ireland (McCarthy *et al.*, 2007) also pointed to the segmentation of the population regarding their food safety knowledge. Four major groups – those “at risk”, “food safety conscious”, “food science knowledge deficient” and “informed” – could be identified

Thus, risk communication has proven to be a key element in public perception of food safety in recent years and deserves continuous attention both from authorities and from scientists.

Control of contamination

Kan (2002) stated in his review that prevention and control of contaminants and pesticides in the poultry production chain requires:

1. Knowledge of the sources of contamination and their consequences
2. Knowledge of the possibilities to exclude these sources from the system or to reduce their effect.

Preventive measures are preferred over “end-of-pipe” approaches due to (cost) effectiveness (Kan, 1994).

Hazard Analysis by Critical Control Points (HACCP) is a systematic approach to the identification, assessment and control of hazards in the food chain. Ropkins et al. (2003) were one of the first to extensively describe the templates to be used for the control of organic chemical hazards in food production. They discriminate between applied chemicals, accidental chemicals and background chemicals and this distinction will be followed in this paper.

Applied chemicals are generally absent in the organic poultry production but feed additives and veterinary drugs are used in keeping hens in conventional systems. Accidental chemicals e.g. cleaning materials, disinfectants and machine lubricants can be used in both organic and conventional production and background chemicals are also a threat to all production systems. Ropkins et al (2003) subdivide the background chemicals for beef production into airborne, waterborne, vegetation and soil contaminants. For poultry in general, housing related contaminants seem to be also of importance and vegetation should be expanded to include feed. Both Ropkins et al (2003) and Kan (2002) point out that applied chemicals are the ones likely to be controlled by a HACCP approach. Accidental and background chemicals are rarely considered by a quality control system and hard to be controlled by HACCP as intervention strategies are generally lacking. Blüthgen (2006a,b; 2007) has in a series of papers eluded on this topic, especially in relation to milk production in Germany.

Dioxin residues in eggs and possible sources of dioxins

Data from several countries are now available that indicate differences in levels of dioxins and dibenzofurans in eggs produced by hens from different management systems. The number of samples has often been restricted, but a certain overall trend does certainly exist.

The data and possible sources have been reviewed by both Kan (2005) and Schoeters and Hoogenboom (2006). More focused reports are the Egg report (DiGanghi and Petrlík, 2005) and recent data from France (Thébault, 2005) and Belgium (van Overmeire *et al.*, 2006). Zhao et al. (2006) reported that in their study in China the PCB and dioxin congener pattern in eggs was not similar to that of the soil, worms and grass. No clear suggestion for this deviation from the generally observed trend was provided.
In different countries thus, the small scale, organic or free range production system seems to add an extra risk for dioxin contamination of eggs. A clear explanation for this phenomenon is not always available.

Three major sources causing dioxin residues in poultry have been described so far:

1. Pentachlorophenol (PCP) related dioxin residues in wood and derived products.
2. Residues in soil mainly caused by deposition of airborne residues caused mainly by (incomplete) industrial combustion processes. However man-made fires are a possible source too.
3. Certain clay minerals e.g. kaolin or ball clay

Landfills where PolyChlorinated Biphenyl (PCB) containing materials have been dumped, are however also recognized as a major source (Hansen and O'Keefe, 1996) Till now they have not been connected to residue problems in poultry. River sediments have also been found to be a potential dioxin source in both the US (Vorhees *et al.*, 2003) and Europe (Schulz *et al.*, 2003). Also for this source a connection with residue problems in poultry has not yet been reported. “Kieselrot” (red slag); a residue from a copper production process was found to contain considerable amounts of PCDD/F. The bioavailability of PCDD/F *in vitro* in this material, which is generally used as coverage for pavements and playground was found to amount to 60 % (Wittsiepe *et al.*, 2001). Poultry being in contact with this material, could thus well display higher dioxin levels. Attention is generally focused to oral absorption but absorption of lipophilic compounds through the skin or the airways should not be excluded (Holmes *et al.*, 1969; Fishwick *et al.*, 1980; Qiao *et al.*, 1997; Rohrer *et al.*, 2003; Imsilp and Hansen, 2005).

Likely causes for dioxin contamination of eggs

Feed, soil, wood treated PCP etc are possible causes for dioxin contamination of eggs; it remains to be proven that they are also the likely causes. The second question is; do the dioxin levels in eggs drop, if you remove those sources from the system?
Air et al. (2003) state: there are many questions and only some answers. They have found that some poultry holders show consistently high or moderate levels of contamination, while others show great variation in their levels. They find soil contamination to be a relevant contributor to PCDD/F levels in eggs, but also reported similar dioxin levels in eggs from two pens with a considerable difference in soil contamination (40 vs 148 pg/g TEQ). Similarly the large Dutch inventory (Brandsma *et al.*, 2004) could unfortunately give no clear clue on the cause of the problem. Some statistical relationships between the many variables measured were found; e.g. small farms had a higher chance than large farms to produce eggs with high contents. Flocks with high dioxin levels also had higher ash content in the manure suggesting increased soil intake. A clear causal relationship did not emerge from the study. Some of the possible solutions like (reduction of) soil intake and reduction of time outdoors, were tested in an intervention study in late 2004, but no clear reduction in dioxin levels in eggs was obtained (A. Kijlstra, personal communication). Air et al. (2003) state that time spent outdoor and picking might be an important item in determining dioxin contamination. The amount of soil or sand taken up by hens was still unknown. For that reason, we included up to 30 % sand in a layers ration (in pellet form) and fed it to young layers for 5 weeks. The hens were, much to our surprise, able to increase their feed intake to (almost) fully compensate the dilution brought about by the sand inclusion. Hens consumed up to 45 grams of sand per day (van der Meulen, Kwakernaak, Kan, 2007, in press). Thus uptake of considerable amounts of soil while maintaining good productivity seems possible.

Experimental studies on carry-over of dioxins

Older studies have been reviewed previously (Kan, 1994; 2005). Hoogenboom et al. (2006) carried out carry-over studies with dioxins in laying hens. The first study with five different dioxin levels in the feed, demonstrated that a level of 0. 750 ng TEQ/kg in feed (the current tolerance) led within two weeks to levels in egg fat above 3 pg/g (the current tolerance). This study consisted of 56 days exposure to contaminated feed and 56 days withdrawal on uncontaminated feed. The dioxin residues in egg and body fat showed a dose response relationship to those in feed. The data were used to construct a PBPK model describing the pharmacokinetics of dioxin residues in laying hens (Van Eijkeren *et al.*, 2006). Steady state in egg residues was not yet obtained after 56 days. The second study tested carry-over of dioxins from two contaminated soils included at 10 % in the feed, to eggs. Residues increased much faster in this trial and the dioxins were almost fully bio-available. Three different binders added to the feed were tested for their effect on dioxin residues in eggs. Unfortunately no effect of the binders on dioxin levels in eggs was observed, as predicted by the review from Kan (1994). Pirard and de Pauw (2006; 2007) have studied carry-over of chlorinated dioxins and furans as well as brominated diphenyl ethers in laying hens. Clearly carry-over of these persistent organohalogen compounds from feed to egg was demonstrated. The low egg laying percentage from their hens and the rapid decline of production after the experimental start, however, throws doubts on the numerical relationships between residues in feed and egg to be calculated from their trial.

Recently Hoogenboom et al (unpublished observations 2007) included a dioxin contaminated soil in a layers diet at a rate of 20 %. To some test diets, binders were added and the diets were fed for 84 days to groups of laying hens. The hens largely stopped laying after 8-10 weeks on the experimental diets and the hens showed a decalcified skeleton at autopsy (at day 84). Probably the high iron content of the soil (20 g/kg) resulted in binding of most of the dietary phosphorous and thus in cessation of lay and a rachitic type status of the hens. Nevertheless, it was quite clear from the residue levels in the preceding period that the binders did not result in lower dioxin levels in the eggs, as suggested by some field trials.

Other contamination sources for (lipophilic) compounds

Coating material of (concrete) farm silos has shown to be a possible source of PCB's (Willett *et al.*, 1985). Leaching from the coating to the material contained in the silo, especially during silage formation, was shown to occur. Another unexpected PCB source was a vapor seal being part of the insulation material on the ceiling of a turkey house, which decomposed over time, fell down and was taken up by the animals (Hansen *et al.*, 1989). Joint sealants in buildings have also been identified as a hitherto overlooked and possible diffuse PCB source (Kohler *et al.*, 2005). Mineral oil hydrocarbons – present in waste oils – have also been found in both feed and eggs (Grob *et al.*, 2001). Thus, proper separation of pure animal or plant derived fat and oil from general fat containing wastes is not only important in relationship to PCBs and dioxins as pollutants. Acrylamide, not only used in wastewater treatment but also formed during heating of potatoes and potato products such as chips, also shows carry-over from feed to egg (Kienzle *et al.*, 2005)

The spraying of propoxur containing formula in poultry houses (not on animals) to combat the poultry red mite (*Dermanyssus gallinae)* did result in violative residues in eggs (> 50 µg/kg) for some days after administration especially in battery housing (Hamscher *et al.*, 2003).

Drugs and coccidiostat residues

The incidence of gastrointestinal helminths in free-range and backyard poultry was higher than in deep litter systems (Permin *et al.*, 1999), but increased use of anti-parasitics or higher incidence of residues has not been reported.

Carry-over of coccidiostats and veterinary drugs from feed and water to eggs has been already described by Kan and Petz (2000). Mortier et al. (2005) have recently demonstrated this phenomenon again for diclazuril, robenidine, halofuginone, nicarbazin and narasin. McCracken and Kennedy (2007) showed very recently that not only yolk and albumen may contain drug residues. They even could detect nitrofurans residues in the eggshell.

The persistence of nicarbazin in layers, eggs and manure depending also on housing conditions has been studies by several scientists. Friedrich et al. (1984) fed laying hens during 7 days feed with 129 mg/kg nicarbazin being the normal dose to give to young birds to prevent coccidiosis. The hens in cages excreted the nicarbazin via both eggs and faeces during and rather short after exposure. Very small amounts remained detectable for quite some time in the yolks. The hens on deep litter were divided at five days after cessation of the administration in two groups; one group remaining on the old litter and one being transferred to clean unshed litter. The excretion in faeces and eggs on the clean litter showed a decline similar to the eggs from the hens in cages. Residues in faeces and eggs from hens on the old litter remained high for weeks. These results clearly prove the stability of nicarbazin (or one active component DNC) in litter and the recirculation of DNC from litter- probably through oral ingestion of litter – to the hens. Administration of 2 mg/kg nicarbazin in feed during 29 days gave similar results (Friedrich *et al.*, 1985).
The importance of litter as a (re)contamination source has also been demonstrated for meticlorpindol (another coccidiostat) in laying hens trials (Hafez *et al.*, 1988). They further demonstrated like Schwarzer and Dorn (1987) did for chloramphenicol, that administration of drugs to replacement hens till about two weeks before the start of lay can lead to detectable residues in the first eggs. Thus, caution is required in exposing replacement hens intentionally or unintentionally to any unwanted substances in the period shortly before the start o egg laying. The explanation is most likely, that yolks are already developing at that time point and the chemicals can be deposited and trapped in those developing yolks. This leads afterwards to detectable residues.

Other chemicals
Heavy metals like cadmium and lead, mycotoxins and polycyclic aromatic hydrocarbons (PAH) do occur in animal and the animal surroundings. Their physiological behavior in animals has been discussed earlier (Kan, 1994). Van Overmeire et al. (2006) reported higher levels of Tl, Pb and Hg in eggs from small scale private owners than in eggs from industrial producers. In our study with soil contaminated not only with dioxins but also with PAH and lead, we have not observed any carry over of parent PAH compounds or Pb from feed to eggs. (Hoogenboom et al., unpublished observations 2007).

Conclusions
Poultry in outdoor farming systems is more exposed to infectious agents and chemical contaminants than birds housed in barns.
Higher dioxins levels in eggs from some outdoor systems than from indoor ones corroborate this. Both general and local environmental pollution with dioxins are probably responsible for it.
Increased incidence of diseases due to more exposure to infectious agents from the environment has been predicted and sometimes found for outdoor systems. An increased disease incidence could lead to more use of veterinary drugs and more or higher drug residues in products of animal origin. Reports substantiating this assumption have not (yet) been published.
Increased attention for risk management in the design and operation of outdoor systems seems appropriate.

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Keywords: residues, eggs, dioxins, soil contamination, heavy metals