CHLORAMPHENICOL AND HUMAN HEALTH

The science of low-level exposures to chloramphenicol – the Vega product

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CONCLUSION

The ppb-levels of chloramphenicol (CAP) monitored in the product under review poses no risk whatsoever for consumers over a lifetime of exposure. The risk of daily exposure to the beverage containing 50 g of the product contaminated with 1.3 µg CAP per kg product, accepting the very conservative linear non-threshold model for genotoxic carcinogens, would be a factor 1000 to 5000 lower than the MTR (that is one additional case of cancer in a population of one million people). Moreover, CAP has been shown to have a natural background detectable by the latest analytical equipment that is moreover traceable in some food products. Below we will elaborate on these unambiguous conclusions extensively and with ample reference to peer-reviewed literature.

INTRODUCTION

Setting scientific and policy standards that benchmark the benefits and risks of products intended to be consumed is of great consequence for industry, policymakers and, consumers. The safety of products consumed is more often than not defined as chemical product-safety, meaning that the product consumed is regarded as ‘safe’ when man-made chemicals such as antibiotics and pesticides are absent or only present at very low levels.

One such chemical is the antibiotic chloramphenicol (CAP). This particular antibiotic is banned from food production, other than when used as a human medication. In this paper we will elucidate its origin, drug medicinal and food exposure levels, risks and the manner in which such risks are derived. The particular Vega product under scrutiny here was tested and in three samples the following concentrations were found according to the test report: 0.42, 0.44, and 1.3 µg/kg product.²

Below, we will not discuss the actual production process of the Vega product. Presence and sources of CAP are discussed in general terms with respect to the state-of-art scientific knowledge available in the literature.

CHLORAMPHENICOL – ORIGINS AND USE

CAP is a natural product, as most antibiotics are. Most antibiotics we now know today are derived from nature’s topmost antibiotic producers, the Actinomycetes. These soil-bacteria are ubiquitously found world-wide. To give an impression, the biomass per hectare (100 × 100 m) of the Actinomycetes in topsoil can be as high as 5000 kg.³

The Streptomyces, belonging to the Actinomycetes, account for well over two thirds of these commercially and therapeutically significant antibiotics. Streptomyces therefore are the most important source of antibiotics for medical, veterinary and agricultural use.⁴ CAP is produced by the Streptomyces venezuelae.⁵

Chloramphenicol⁶ was the first antibiotic to be synthetically produced and was shown to be effective against typhoid.⁷ It was widely used in the world against infections and was also used as a

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² These values have been made available to us by Gowling Lafleur Henderson LLP. Values have been determined by Eurofins.
veterinary drug. Nowadays CAP is banned from food-production, specifically in the animal food-production chain, and is hardly used as a human medication, for reasons discussed below, although in Asian countries CAP is still used as a human therapeutic agent such as typhoid. Ophthalmic infections, however, are still treated extensively with CAP.

CHLORAMPHENICOL – (THERAPEUTIC) RISKS

Two types of risks have been identified in relation to medicinal use of CAP. Firstly, aplastic anaemia, a form of anaemia when the bone marrow ceases to produce sufficient red and white blood cells, is the most dangerous effect produced by medicinally used CAP. Its occurrence is extremely rare, albeit fatal and is only observed as a result of therapeutic treatment with CAP.

The total aplastic anaemia incidence estimated by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) is in the order of 1.5 cases per million people per year. Only about 15 per cent of the total number of cases was associated with drug treatment and among these CAP was not a major contributor. These data roughly gave an overall incidence of therapeutic CAP-associated aplastic anaemia in humans of less than one case per 10 million per year, which is a factor 10 below the Maximum Tolerable Risk (MTR) level of 1:10^6 (see below).

In considering epidemiological data derived from the ophthalmic use of CAP, systemic exposure to this form of treatment was not associated with the induction of aplastic anaemia. Because of the limited data available, however, it is unfeasible to determine a genuine medicinal dose-response correlation for the occurrence of aplastic anaemia. The risk of aplastic anaemia is the main reason why CAP fell out of favour as an antibiotic.

Secondly, limited evidence exists for the genotoxic carcinogenicity of CAP in humans exposed to therapeutic doses. CAP is categorised by the IARC (the International Agency for Research on Cancer) as probably carcinogenic in humans; group 2A. However, the available data on the genotoxicity of CAP show mainly negative results in bacterial systems and mixed results in mammalian systems. It was concluded that CAP should be considered genotoxic, but only at concentrations about 25 times higher than those occurring in patients treated with the highest therapeutic doses.

Overall, the risk data on CAP is limited. Aplastic anaemia is extremely rare even when considering direct exposure to high levels of therapeutic CAP. As CAP is hardly used anymore medicinally, no more data is forthcoming thereon. With respect to the genotoxic carcinogenicity of CAP,

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the data are conflicting. As CAP is therapeutically out of favour, both in the human and veterinary field, experimental research on CAP is a thing of the past.

Only the second risk issue—the limited evidence for genotoxicity—was the reason to ban CAP from the food production chain. As remarked by JECFA: 'The Committee was unable to establish an ADI for CAP due to the lack of information to assess its carcinogenicity and effects on reproduction and because the compound was genotoxic in a number of in vivo and in vitro test systems.'\textsuperscript{15} The fact that no ADI (Acceptable Daily Intake) could be established resulted in the following:

- **Lack of scientific data** makes the establishment of a TDI (Tolerable Daily Intake) for CAP not feasible;
- The absence of a TDI, and the subsequent impossibility to establish a maximum residue limit (MRL), in regulatory terms is understood as 'dangerous at any dose', requiring zero tolerance regulation, nowadays translated into explicit levels of concern using the so-called MRPL (Minimum Required Performance Limit) level, that is whatever low concentration levels regulatory laboratories can detect and confirm;
- With the introduction of zero tolerance, a veterinary ban is in place, whereby the listed compounds, when producer’s compliance is achieved, should disappear from the food chain.

However, when zero-tolerance was implemented, analytical equipment then was only capable to detect at the Limit of Detection (LOD) of ppm (parts per million; mg per kg); nowadays LODs are at least ppb (parts per billion; µg per kg) and even lower, obviously depending on analysed chemicals and available equipment (see further below).

Summarising, **CAP is banned for use in food-producing animals** in the European Union (EU)\textsuperscript{16} and in many other countries, including the United States, Canada, Australia, Japan, and China. The ban was implicitly regarded as a legal means to purge the food production chain, and related consumable goods, of CAP.\textsuperscript{17}

**CHLORAMPHENICOL – SOURCES AND ECOLOGY**

Now, with the ban on CAP-use in the food-production chain in place, and medicinal use of CAP almost non-existent (apart from ophthalmic use in the Western world and more all-round therapeutic use in Asian countries), it might be surprising that CAP is still found in products intended to be consumed, including those derived from plant material.

The source of concern might well be the environment (if CAP is not deliberately added to the production process, one way or another). CAP’s usage as a human medicinal antimicrobial could result in its release into the environment through various waste streams by which consumable products may be contaminated during the production phase. Hirsch *et al.*, for instance, did find

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\textsuperscript{15} Note 10.


See further: *Guidance of the CONTAM Panel Scientific Opinion of the EFSA Panel on Contaminants in the Food Chain (CONTAM)*. 2013. European Food safety Authority, Brussels.
CAP in the aquatic environment in Germany.\textsuperscript{18} It was detected in the effluent of one sewage treatment plant and in surface-water at concentrations of 0.56 µg/l and 0.06 µg/l respectively. Lower levels were found in Switzerland, that is between 0.01 – 0.03 µg/l.\textsuperscript{19} In China, concentrations of CAP in wastewaters of two sewage treatment plants were somewhat higher. CAP was detected between 1.73 – 7.91 µg/l.\textsuperscript{20} However, in two other wastewater treatment plants, CAP concentrations were significantly lower; only 0.022 µg/l was found in one treatment plant in May and 0.0058 µg/l in November.\textsuperscript{21}

The ability of CAP, potentially present in the environment as a result of therapeutic use, to contaminate production processes for consumable goods seems limited, however, as the half-life of CAP is fairly short, roughly one to a couple of days depending on the conditions.\textsuperscript{22}

Another environmental source could well be natural in origin. Already in 2003, during the height of a trade-dispute between the European Union and some Asian countries on the presence of CAP in shrimp, we raised the question on the ecological background of CAP in food.\textsuperscript{23} Indeed, we specifically investigated numerous European food products on the presence of CAP and found that at least one product, a certain European brand of white wine, contained 2.7 ppb of CAP. In 2010, unambiguous evidence was published on the ecological background of chloramphenicol in herbs and grass.\textsuperscript{24} In their words: ‘... Among other plant materials, samples of the Artemisia family retrieved from Mongolia and from Utah, USA, and a therapeutic herb mixture obtained from local stores in the Netherlands proved to contain CAP at levels ranging from 0.1 to 450 µg/kg.' This research was followed up in 2013, in which other plant material was found to contain CAP of natural origin.\textsuperscript{25}

The discussion on the presence of CAP in plant material has come to the fore because of the increasing capabilities of analytical equipment. As said, these capabilities have greatly increased over the past decades. What is more, we have entered the realm of atto- (part per quintillion; 10\textsuperscript{-18}) and zeptomoles (part per sextillion; 10\textsuperscript{-21}) of detectable analytes.\textsuperscript{26} Basically, this means that


the zero tolerance level is shifting to ever lower exposure levels. Advances in ‘cleaner’ consumable goods production is thus offset by increased detection capacities, whereby the ecological threshold for compounds such as CAP can be crossed, and in fact has been crossed as shown above.

As the Vega product contains enzymes fermented in the presence of plant materials, it seems plausible that the CAP found therein is from a natural source ultimately exposed to the antibiotics-producing Actinomycetes. Obviously, we have no means to actually verify this hypothesis and rule out (or corroborate) other sources of the (non-natural) therapeutic kind, but the latest research referred to here has made the premise of a natural background quite credible. Be that as it may, what is the residual risk profile of the presence of CAP in the product under scrutiny?

THE RISK PROFILE OF THE VEGA PRODUCT RELATED TO CAP

First, it is important to show the difference between the medicinal exposure to CAP and the exposure as a result of CAP-presence in consumable products as monitored. We will take the 1.3 µg/kg product value as a starting point.

According to Vega, the dose of product used to prepare the final consumable beverage is from 35 g to 41.7 g in 250 ml of water. So, assuming consumers use 50 g of the product, the final beverage would contain 0.065 µg of CAP. Just to emphasise, the dilution in water as such is not an issue here; just the total amount of Vega product used for consumption is relevant. The actual consumed load of product thus determines the intake of CAP.

Assuming that the consumer weighs 70 kg and consumes one such beverage on a daily basis, this would amount to 0.00093 µg/kgbw/day (which is 0.0000093 mg/kgbw/day). Compared to the therapeutic exposure to CAP, which of course cannot be considered a lifetime event, the difference amounts to the following:

<table>
<thead>
<tr>
<th>Therapeutic CAP exposure levels: 25 - 125 mg/kgbw/day</th>
<th>Exposure-level difference</th>
<th>CAP exposure level in product: ± 0.0000093 mg/kgbw/day</th>
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<td>26 881 720 - 134 408 602</td>
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This substantial difference of exposure accounts for the fact that product residual exposure levels of CAP account for negligible risk, as we will see below, even considering such an exposure as a daily event over a period of a lifetime.

The Dutch National Institute for Public Health and Environment (RIVM) calculated the risk of exposure to CAP, applying the conservative linear non-threshold model (LNT) by default used for genotoxic carcinogens (CAP purportedly being genotoxic). The RIVM came to the conclusion that exposure to 1 – 5 µg/kgbw/day of CAP posed a risk to humans at the level of a 1:10^6 added cancer risk, the so-called Maximum Tolerable Risk level (MTR). This means that the regulatory standard for acceptable risk from a carcinogen is one additional case of cancer in a population of one million people. The MTR-level for carcinogens consequently is vanishingly small.

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The analysis done by the RIVM is related to food (shrimp) but of course equally holds for consumable products, such as the product from Vega. It holds because the implicit assumption is made in the risk assessment that the CAP as found in the consumable product is bioavailable to humans when consumed. Thus:

| Applying the conservative linear non-threshold dose-response model for the calculated exposure – that is 0.00093 µg/kg bw/day - the risk of daily exposure to the beverage containing 50 g of the product contaminated with 1.3 µg CAP per kg product would be a factor 1000 to 5000 lower than the MTR. That would amount to a vanishingly and immeasurably small risk (only if the LNT model is justifiable). |

Reiterating, the above calculation is based on the conjecture that a LNT dose-response correlation is valid. This regulatory model holds that for genotoxic carcinogenic substances and ionising radiation, any level of exposure – except for zero – implies a health risk. Only zero-exposure is ultimately deemed to be safe. This so-called ‘one hit’ dose-response model thus maintains that exposure to even one molecule or ionising photon may result in irreversible health damage. Even accepting this conservative model the risk of exposure as assessed here is vanishingly small.

The potential effects of genotoxic carcinogenic substances and ionising radiation at very low-level exposures are however utterly theoretical: they are derived from this model as, of course, actually observing those effects in human populations would be out of the question. The effects, if at all existent, are simply far too small to measure. Indeed, any proper validation of the LNT model has never been accomplished.

This conservative toxicological model, therefore, is scientifically (and philosophically) untenable and coming increasingly under fire. Already in 1996, Goldman noted the absurdity of the LNT model when he linearly calculated the increased risk of cancer, because of increased cosmic radiation, if the entire world population would add a 1-inch lift to their shoes (sic):

> ‘As an extreme extrapolation, consider that everyone on Earth adds a 1-inch lift to their shoes for just 1 year. The resultant very small increase in cosmic ray dose (it doubles for every 2000 m in altitude), multiplied by the very large population of the Earth, would yield a collective dose large enough to kill about 1500 people with cancer over the next 50 years Of course no epidemiological confirmation of this increment could ever be made, and although the math is approximately correct, the underlying assumptions should be questioned. Most of the environmental risks we now face from present or proposed activities probably are of this magnitude, and many of our policies say that prudence requires us to reduce these small values even further. We do not seem to have a realistic process whereby we can uniformly both protect the public health and avoid seemingly frivolous prevention schemes.’

This is the basic scientific and regulatory assumption, even when people are actually exposed, under normal conditions, to doses several thousand fold or even several hundred thousand fold

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lower than the tested animals,\textsuperscript{31} say, for example, through consumable products. What is more, a
dose of various carcinogens to humans associated with a \textit{de minimis} risk of cancer (< 1 can-
cer/million/lifetime exposure; the MTR referred to above and used in the calculation) would
commonly deliver many trillions of carcinogenic molecules each day for a 70-year lifespan, a
value approaching and at times exceeding some 18 orders of magnitude greater than the so-
called proverbial single molecule.\textsuperscript{32} In all intents and purposes, the LNT model grossly overestimates risks of exposure to low levels of chemical compounds such as CAP, either man-made or nat-
ural.\textsuperscript{33} Overall, the LNT is an unacceptable model ill-suited for determining risk of exposure at
low levels.

The debate on the LNT model is intensifying, and for instance the French Academy of Sciences
and the French National Academy of Medicine have raised serious doubts as to the validity of the
LNT model, effectively abandoning the model.\textsuperscript{34}

Forestalling exposure to chemicals at levels such as discussed here is a flawed and unsustainable
approach when considering chemical product-safety in light of the increasing capabilities of
science and technology. It augments uncertainty with regards to the presence and sources of in-
creasing numbers of detectable chemicals, opens the door to ecological background concentra-
tions of compounds fallaciously regarded as man-made only,\textsuperscript{35} and proliferates public anxiety
when a ‘new’ chemical is detected at ever-lower levels, \textit{whereby toxicological relevance is ig-
nored.}

\textsuperscript{31} Calabrese E.J., 2009. The road to linearity: Why linearity at low doses became the basis for carcinogen risk assessment. \textit{Archives of Toxicology} \textbf{83}: 203 – 225.
\textsuperscript{32} Calabrese, E.J., Cook, R.R., Hanelkamp, J.C. 2012. Linear No Threshold (LNT)—The New Homeopathy. \textit{Environmental Toxicology and Chemistry} \textbf{31}: 2723


\textsuperscript{35} See e.g.: Öberg, G. 2002. The natural chlorine cycle – fitting the scattered pieces. \textit{Applied Microbiology and Biotechnology} \textbf{58}: 565 – 581.