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Environmental Repercussion of the consumption of antibiotics

Environmet

How antibiotic waste gets into the natural environment and what its effects are.

Antibiotic waste from human consumption, covered by no regulations or legislation, is continually being spilled into the environment. It ends up in wastewater treatment plants where it might resist all treatment and expand further through farming use of the sewage sludge as a soil conditioner. These substances are still active and produce diverse effects on the receptor environment (surface water, underground water and soil) through long term exposure. The study of their environmental behaviour and potential effects is regulated only by drugs legislation. Application for authorisation and registration of the drugs by European agencies includes the obligation of assessing the drugs' environmental risk before marketing authorisation. There is therefore a need for conducting environmental studies to monitor drug waste, broken down into various environmental compartments, and also to weigh up its potential effects and any environmental risk.

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The environmental problem of the consumption of antibiotics

A vast array of anthropogenic chemical substances now reaches the environment. Though covered by no current legislation this waste is continually being spilled into the environment due to the mass consumption of these products. We are speaking here of pharmaceutical specialities. According to the annual report issued by the General Council of Pharmaceutical Associations (*Consejo General de Colegios Oficiales Farmacéuticos*) the national healthcare drugs market (i.e., drugs taken on prescription) represents over 90% of sales in Spanish chemists[1]. The most commonly consumed drug types of all those registered in Spain (Spanish Agency of Drugs and Healthcare Products - *Agencia Española de Medicamentos y Productos Sanitarios:* AEMPS) are anxiolytic and hypnotic agents, with an increase of almost 103% in the period running from 1997 to 2006, followed by the non steroidal anti-inflammatory drugs (ibuprofen, diclofenac and aclofenac) and in third place the antimicrobial agents (Figure 1).

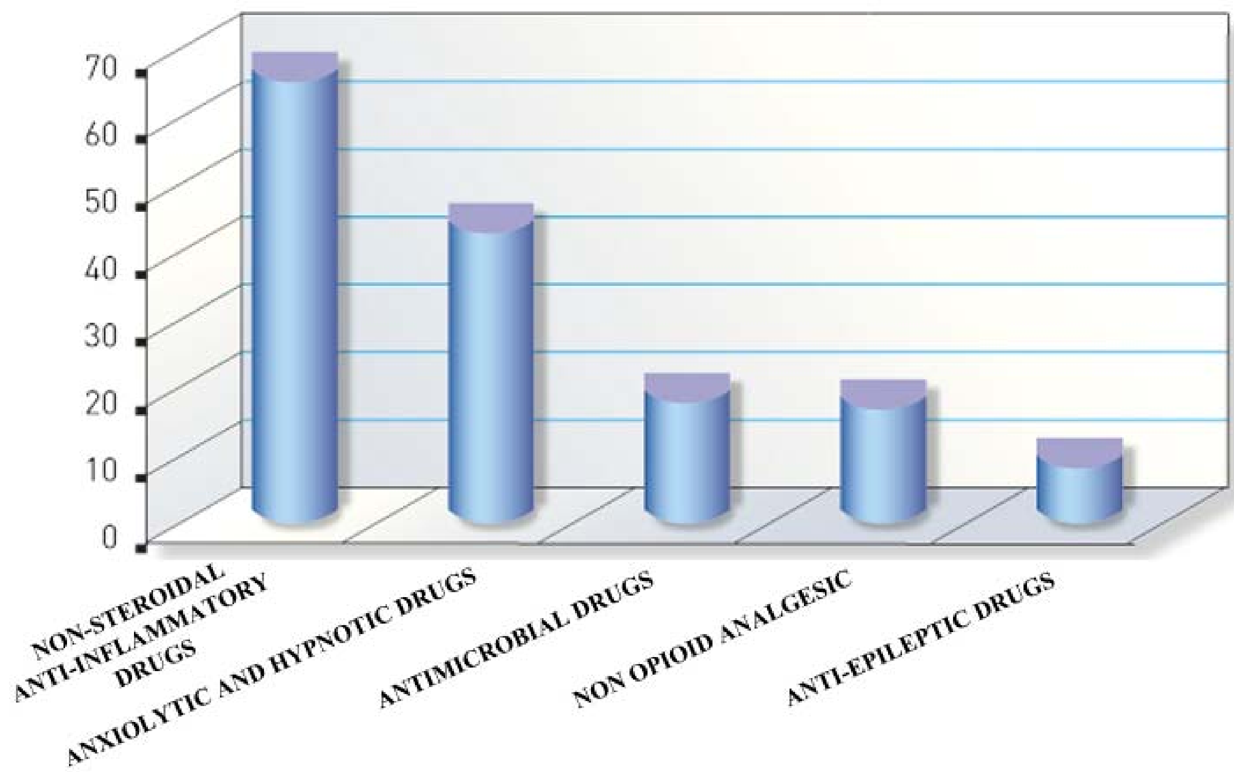

Figure 1. Most frequently consumed drug groups within the Spanish Health Service[2].

Of a total of 200 antibiotics approved at national level, 52% is meant for human consumption (therapeutic and prophylactic use, 10% in hospitals and 90% in the community) and the remaining 48% is for veterinary use (therapeutic and prophylactic use). Spain is one of the European Union's biggest consumers of human-use antimicrobials[3], ranking third according to the European Surveillance of Antimicrobial Consumption. Its population consumes, above all, a wide range of antibiotics, which are the drugs with the biggest impact on the development of resistance.

In 2009 penicillins accounted for 62.6% of antibiotics consumption. The most widely used subgroups after penicillins are quinolones (12.2%), macrolides (9.7%) and cephalosporin (7.9%) (Figure 2).

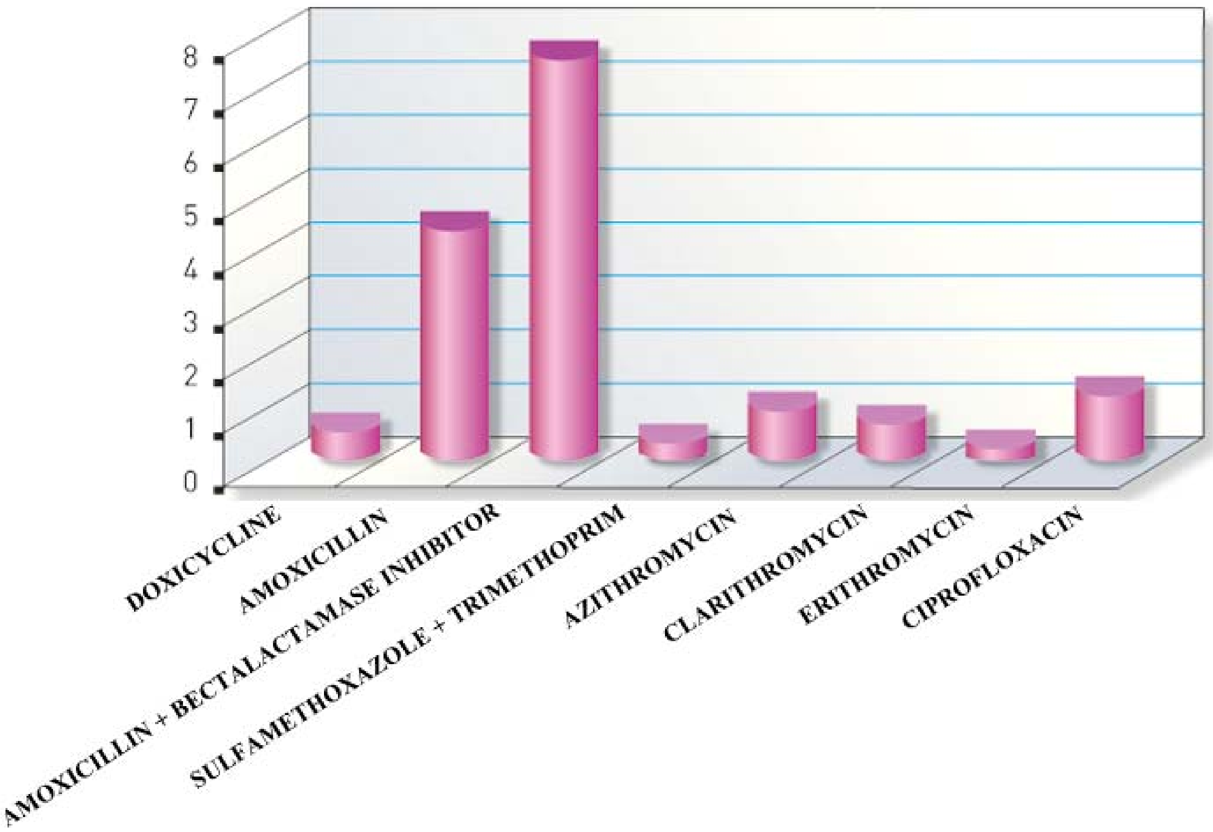

Figure 2. Consumption of antibiotics in Spain in 2009 under the Spanish National Health Service, expressed in daily doses defined by 1000 inhabitants per day(1).

How they get into the environment

The main vector through which antibiotics reach the natural environment is wastewater from domestic therapeutic use Other lesser vectors include the uncontrolled dumping of domestic and hospital waste of unused antimicrobials or outflow from pharmaceutical factories[4]. In general, once taken internally and after being metabolised, they are excreted mostly a an original parent compound and, to a lesser extent, as metabolites[5] . Through wastewater these excretions end up i Wastewater Treatment Plants (WWTP). The amount of antibiotic waste present at any one time will depend on the frequency and amount of its prescription (consumption patterns), the excretion pattern of the original compound, it metabolism, the affinity of the drug or its metabolites for absorption by organic matter (physicochemical properties of the active principle) and the metabolic transformation capacity of the WWTP's microorganisms[6].

A vast array of anthropogenic chemical substances, not covered by current legislation, gets into the natural environment, such as the waste from pharmaceutical products

After treatment in these wastewater plants the effluent, and with it the antibiotic waste, is directly discharged into rivers and other surface wate masses and also their sediment[7]. Sewage sludge may also contain this waste which can then be spread on the land under the current practice of reusin sludge on farming[7,8].

Sewage Sludge

The land receives this drug waste through the reuse of sewage sludge as an organic soil conditioner. In recent years there has been an increase in the production of sewage sludge after implementation of Directive 91/271/EEC[9], which obliges al built-up areas with a population of over 2000 to treat local wastewater. Production of sewage sludge has therefore grow by 41% in Spain since implementation of the European Directive into Spain's body of law by Royal Decree Law (*Rea*

Decreto Ley) 11/1995[10] and application thereof by Royal Decree (Real Decreto) 509/1996[11] up to 2007. We can ther appreciate how this circle is closed if we bear in mind the management model of these sub-products, one of the solution being the agricultural reuse of this sludge [12, 13]. The composition of this sludge, albeit variable, makes it a source o organic matter and fertilising elements for farms. In Spain in 2007 66.7% of sewage sludge was used on farms[14]. The agricultural use of this sludge does run the risk of environmental pollution, especially of the soil. The application dose has to be fixed in light of the soil's agronomic characteristics, the permitted build up of metals in the soil and nutrient needs o the crops[13]. There is no reference to the presence of waste from organic contaminants of anthropogenic origin and, ipso facto, no reference either to antibiotic waste.

Much of this waste is persistent and resists the sewage treatment process. Even waste of a lower persistence, however might have the same exposure potential in the end because its higher speed of transformation/elimination might be offse by its much faster input rate into the environment.

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Existing studies on the presence of drug waste has tried to identify this waste in sewage effluent, as the main emission vector into the environment after its human consumption[15]. As a general rule the sewage-farm sludge line is not taken into account but it is precisely this sludge that, depending on the physicochemical properties of the various groups of antimicrobials, might to a greater or lesser extent be retained in the environment[16]. Some studies published by the scientific community have shown that antimicrobials like sulfamethoxazole and roxithromycin might be removed from effluents with an efficacy of 40 and 65%, respectively[15]. A fraction might be broken down by the treatment but most ends up in the sludge line which is generated as a subproduct during the wastewater treatment system Antibiotic waste with a strong affinity for matter in suspension has been recorded, at concentrations ranging from 1.4 to 2.4 mg/kg dry weight of treated sludge. Other antimicrobials are also turning up, like sulfamethoxazole, trimethoprim and azithromycin[17,18]. In Spain environmental monitoring is focusing on the most heavily consumed drugs and is geared mainly towards the recovery and recycling of WWTP effluent and surface water[19]. There are few studies backed up by Spanish scientists for monitoring this waste in sewage sludge or its impact after reuse on farming land.

The figures show a great heterogeneity bound up with a host of factors such as population density, consumption patterns o type of wastewater treatment[20]. The antimicrobials that turn up most often in sludge are the macrolides (32-195 µg/kg) fluoroquinolones (40-886 µg/kg) and sulphonamides (0-31 µg/kg)[17, 18, 20]. They have been detected in farming land a maximum concentrations of 500 µg/kg[21] due to build-up in the soil. Tetracyclines, for example, have been recorded a concentrations of 86 -118 mg tetracycline/kg and 4.6-7.,3 mg chlortetracycline/kg[22, 23].

Environmental effects of the presence of antibiotic waste

Although the concentration of drugs in sludge may be very low in terms of µg per kg, many of them remain active in the environment, especially the antibiotics. Furthermore, since they are not dangerous to public health, like pesticides, they have not been subjected to the same screening to find out their adverse effects on the environment. These compounds have been slowly but steadily discharged into the environment for decades, so the organisms of the environment concerned suffer chronic exposure to them.

> *Once taken and after being metabolised they will end up in the natural environment in low concentrations through the vectors of treated wastewater and sewage sludge*

One of the main problems that might be building up as a result of this chronic exposure to antimicrobials is the induction of resistance among anthropogenic bacterial populations and the natural populations of the ecosystem concerned This might be happening within the treatment system itself or once they have been released into the environment[24]. Bacteria, therefore, whether pathogens or otherwise, may well be serving as reservoirs of resistance genes, thus contributing to the evolution and dissemination of this resistance in the environment. From this point resistant bacterial populations might then pass on genes responsible for the resistance, often inserted into mobile elements (plasmids, transposons and integrons), individually or grouped within these structures (multi-resistances), to other microorganisms, pathogens or otherwise, sharing the same environment. From here they pass on to animals and food and from there back to man, generating a vicious circle becoming more hazardous with each cycle[6, 7].

Various international organisations have raised the alarm about the appearance of bacterial and antimicrobial resistance[25]. In the last 10 years several initiatives have also been set up, like the European Antimicrobial Resistance Surveillance Network (EARSS), the EU Reference Laboratory for Antimicrobial Resistance (CRL-AR), the Scientific Advisory Group on Antimicrobials (SAGAM-EMEA) or the Working Group on Antimicrobial Resistance (OIE, FAO, WHO). At national level mention must be made of the Veterinary Surveillance Network on Resistance to Antimicrobials (*Red de Vigilancia Veterinaria de Resistencias a Antimicrobianos*). The latest breakthroughs in this field have enabled antimicrobials to be prioritised, on the grounds that they are critical for preserving health, both of people and animals (cephalosporins, macrolides, penicillins, quinolones and aminoglycosides). There is now a perceived need for setting up measures to mitigate antimicrobial resistance spread and hence preserve their therapeutic efficacy. These measures would need to include a clinical practice guide of widespread consensus incorporating rational antibiotic-use criteria, such as the local prevalence of resistance[3].

The surveillance of bacterial resistance has mostly involved isolated microorganisms of clinical samples but it is also important to study isolated bacteria of environmental samples to find out their possible role as a reservoir of resistance-coding genes[26]. The resistance-building mechanism in the environment has hardly been studied as yet. Recently, in quinolone-contaminated rivers, bacteria have been detected with genes conferring quinolone resistance[26, 27]; they have also been found in the soil[28]. A study has also been made of the relation between resistant bacteria in rivers and in WWTP effluent[26].

Until now, however, there has been no positive evidence to show whether or not the low levels of antibiotics found in the environment exert any pressure on environmental bacterial populations or the dispersion of resistance genes[29].

Need for a field study

Although the risk studies carried out in other countries suggest that the levels of antimicrobials in the environment do not pose a risk to human health[6], the truth is that no specific study has been conducted in Spain to ascertain whether or not this is true. There has not even been any exhaustive study of soil levels after farming reuse of the sludge.

> *Although the concentration of drugs in sewage sludge may be very low, many of them remain active in the environment, especially the antibiotics*

At national level it is an emerging subject that has been studied in certain one off areas of Spain[30]; there is now a need to flesh out this information with more data from other areas. The aim of this study is therefore to analyse, firstly, the presence of human-use antibiotic waste in sewage sludge and, secondly, its potential for inducing resistance in the main bacterial populations considered in human medicine.

Study Objectives

The main objectives of this research project are: a) to establish the emission levels in sewage sludge as the main source of human-use antimicrobial waste, reaching the environment indirectly through recycling on farmland; and b) evaluate the emergence or increase of resistance in the bacterial populations of sewage sludge and the land it is spread on.

Methodology

Selection of the antimicrobials to be studied

The antibiotics to be studied were selected in view of three variables: firstly, the degree of consumption in human medicine, secondly their capacity of resisting the wastewater treatment process or persistence and thirdly the level of bacterial resistance they show according to the figures obtained by the Enterobacteria Service (*Servicio de Enterobacterias*) of the National Microbiology Centre (*Centro Nacional de Microbiología*).

The initial selection was: from the tetracycline group, tetracycline; from the sulphonamides group, sulfamethoxazole; from the quinolone group, ciprofloxacin, and from the betalactamase group, amoxicillin or, in default thereof, clavulanic acid (the beta-lactam ring of amoxicillin is readily hydrolysed by beta lactamases whereas clavulanic acid is resistant to the action of these enzymes).

Identification and selection of emission points. Sample taking

Sewage sludge from WWTPs has been identified as an emission source. The study area was the region of Castilla y León

Samples were taken from two WWTPs: one WWTP designed to serve a P.E >15.000 and another WWTP designed to serve a P.E <15.000. The samples were taken in July 2011. The sampling plans were drawn up under the water quality standard UNE EN ISO 5667-13: 1998[31].

Sample Preparation

Samples were prepared according to the recommendations laid down in EPA 1694: Pharmaceuticals and personal care products in water, soil sediment and biosolids by HPLC/MS/MS[32], with some tweaks in light of other scientific reference works[33, 34].

Chromatography

The chromatograph used was an HPLC 2695 (Waters) with column heater, automatic injector and mass spectrometer 3100 MS (Waters). The column used was a Gemini-NX C18 (5 µm 110 150 x 4.6 mm) by Phenomenex. Chromatographic and detector conditions were based on the EPA 1694 method [33] for separation of the antibiotics from the groups 1 (ciprofloxacin and sulfamethoxazole) and 2 (tetracycline).

The quantification ions and retention times are shown in table 1.

Table 1. Antibiotics used in the study, ions used for their quantification and their retention times in the matrix used

	m/z	tR
Tetracycline	445,2	13,75
Ciprofloxacin	332,2	15,14
Sulfamethoxazole	254,2	19,36

The waste quantification equipment was calibrated by means of calibration curves for each antibiotic under study using the doped matrix addition method, at different concentrations in geometric progression and selected in view of the expected concentration range to be found in the environment.

Generic resistance screening. Amplified identification and genetic environment of the resistance

The sludge samples were taken from the same two WWTPs as above. First the sludge was homogenised by collecting it in five points and preparing 1:10 and 1:100 dilutions. Two hundred µL of this homogenised sludge was sewn on Mac Conkey and Mac Conkey agar plates supplemented with antibiotic (nalidixic acid, sulphonamides, tetracycline and cloramphenicol). Before reading off the results it was kept in incubation at 37º C for 24 hours.

For colony identification purposes, given that their morphology is so similar, it was decided to identify one colony from each one of the MacConkey agar plates.

Molecular identification was made based on amplification and sequencing of the 16S ribosomal DNA. Once the sequence had been obtained, the identity was checked by using BLAST.

Results and Discussion

The levels of antibiotic waste in the sludge studied herein fall below the detection limit of the analytic method employed for each one of the antibiotics under study (Table 2).

Table 2. Results obtained in the sludge studied. Detection limits, quantification limits and recovery percentages for each antibiotic

Antibiotic	LD (mg/Kg)	LQ (mg/Kg)	% Recovery rate	Sludge 61	Sludge 62
Tetracycline	0,585	1,123	66	< LD	< LD
Sulfamethoxazole	0,0033	0,011	100	< LD	< LD
Ciprofloxacin	0,567	1,888	85	< LD	< LD

The absence of this antibiotic waste may be due to a fall in the consumption of human-medicine antibiotics during the summer months[30, 35, 36]. Antibiotic use in Spain is highly seasonal, peaking in the winter months. This seasonal lopsidedness applies above all to antibiotics used for infections of the respiratory tract, such as penicillin, cephalosporin and macrolides[3]. The absence of tetracycline and sulfamethoxazole in the sludge may be the result of different consumption patterns, as we pointed out in the introduction (Figure 2).

The absence of antibiotic waste in the sludge studied is due to seasonal variations in the consumption of these drugs

Few studies have been conducted to date on the presence of antibiotic waste in sewage sludge. Environmental monitoring studies of this type of waste have been carried out hitherto on WWTP influent and effluent, confirming its absence or describing its levels in said effluents[18, 20, 37]. On many occasions, however, the lack of any sign of this waste in the effluent does not necessarily mean it has been removed altogether, since it might still be found in the sludge. Depending on its physicochemical properties it has been found to have a greater or lesser affinity for organic matter, cations, metals etc. And depending in turn on these specific characteristics, drug waste will be distributed along the WWTP treatment lines either in the water line or the sludge line.

For example the antibiotics selected for this field study in general show a high absorption capacity into the sludge, this being the main vector for their removal from the treated wastewater[6, 37]. Ciprofloxacin has been found at levels of 1.4 - 3.1 mg per kg dry weight, levels of 86 -118 mg tetracycline per kg of tetracycline, and for sulfamethoxazole levels that might reach 31 μg per kg dry weight in sludge[17, 18, 20, 22, 23, 38]. More interesting still is their persistence in sludge-treated soil[37] , where they build up in surface soil layers with limited mobility and little chance of reaching the phreatic water.

Resistance studies have shown negative results for the species and groups of antibiotics included in this study. One of the reasons for this might be the high-temperature treatment of sewage sludge for its reuse in due accordance with microorganism-related sanitary rules[13], confirming compliance with the microbiological criteria. The only colony growth observed corresponded to the bacteria *Pseudomonas pseudoalcaligenes*. This bacterial species forms part of the biofilms used in the biological water-purification processes. There have been few studies of the role these bacteria may play once they reach the natural environment, above all in the resistance to antibiotics and the spread of this resistance. Phenotypic resistance to antibiotics in biofilm bacteria would be positive from the point of view of the treatment system, which could then directly protect the receptor environment from the release of antibiotics[29]. Conversely, however, once in the environment they might favour resistance spread[39].Nonetheless it has not been possible to ascertain to date whether or not the extremely low concentrations of antibiotics in the environment exert any pressure on environmental bacteria populations or the spread of resistance genes[29].

Conclusions

Several conclusions can be drawn from the work carried out in this study. Firstly, that the sludge studied does not show detectable antibiotic levels with the procedure employed herein. Furthermore, this lack of antibiotic waste might be bound up with a seasonal reduction in antibiotic consumption; i.e., the amount of antibiotic waste that might reach the soil as a result of farming sludge reuse as organic soil conditioner depends on annual variations in the consumption of these drugs. This could be a variable to be taken into account in planning the management of sewage sludge, using it as farming fertiliser at times of the year when this sludge has the lowest amount of antibiotic waste.

The information generated in field studies could serve as a scientific base for decision taking by the working groups of drugs agencies and by sludge management institutions

Moreover, field studies similar to the one presented herein offer very important information on actual environmental concentrations of antibiotics in the various environmental compartments and also their distribution and dynamic in the environment. This information could serve as a scientific base for decision taking by the working groups of the European drug agencies, for the purpose of weighing up the environmental risk (assessment of the environmental risk) of the drugs with marketing authorisation in the pipeline. Any pharmaceutical firm submitting a request for registration and marketing authorisation to the corresponding national drugs agency is bound to present at the same time an assessment of the environmental risk[40] conducted by experts in due accordance with technical guides or documents[41]. On many occasions estimation of this risk in accordance with the guides, calls for a field study to determine the real situation of this drug after its arrival in the natural environment in the environmental conditions under which it is being used.

Furthermore, this study represents a groundbreaking step in the field of bacterial resistance and environmental waste of antibiotics, since it is the first time that an attempt has been made to relate both factors, thus filling in an information gap that we have previously seen to exist.

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TO FIND OUT MORE

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